

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
24 October 2002 (24.10.2002)

PCT

(10) International Publication Number
WO 02/083180 A1

- (51) International Patent Classification⁷: **A61K 47/48**
- (21) International Application Number: **PCT/EP02/03591**
- (22) International Filing Date: 25 March 2002 (25.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
01201095.5 23 March 2001 (23.03.2001) EP

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

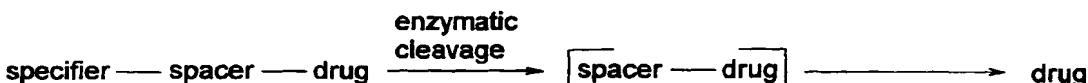
Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ELONGATED AND MULTIPLE SPACERS IN ACTIVATABLE PRODRUGS

WO 02/083180 A1



(57) Abstract: This invention is directed to prodrugs that can be activated at the preferred site of action in order to selectively deliver the corresponding therapeutic parent drugs to target cells or to the target site. This invention will therefore primarily but not exclusively relate to tumor cells as target cells. More specifically the prodrugs are compounds of the formula $V-(W)_k-(X)_l-A-Z$, wherein: V is a specifier; $(W)_k-(X)_l$ -A is an elongated self-elimination spacer system; W and X are each a 1,(4+2n) electronic cascade spacer, being the same or different; A is either a spacer group of formula $(Y)_m$ wherein: Y is a 1,(4+2n) electronic cascade spacer, or a group of formula U being a cyclisation elimination spacer; Z is a therapeutic drug; k, l and m are integers from 0 (included) to 5 (included); n is an integer of 0 (included) to 10 (included), with the provisos that: - when A is $(Y)_m$: $k+1+m \geq 1$, and if $k+1+m = 1$; - when A is U: $k+1 \geq 1$.

Title: Elongated and Multiple Spacers in Activatable Prodrugs

Cross-reference to related application:

This application is a continuation of EP-A-01201095.5, filed 23 March 2001, the disclosure thereof being incorporated by reference.

FIELD OF THE INVENTION

This invention is directed to prodrugs that can be activated at the preferred site of action in order to selectively deliver the corresponding therapeutic or diagnostic parent moiety to target cells or to the target site. This invention will therefore primarily but not exclusively relate to tumor cells as target cells.

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BACKGROUND OF THE INVENTION

- Lack of selectivity of chemotherapeutic agents is a major problem in cancer treatment. Because highly toxic compounds are used in cancer chemotherapy, it is typically associated with severe side effects. Drug concentrations that would completely eradicate the tumor cannot 10 be reached because of dose-limiting side effects such as gastrointestinal tract and bone marrow toxicity. In addition, tumors can develop resistance against anticancer agents after prolonged treatment. In modern drug development, targeting of cytotoxic drugs to the tumor site can be considered one of the primary goals.
- 15 A promising approach to obtain selectivity for tumor cells or tumor tissue is to exploit the existence of tumor-associated enzymes. A relatively high level of tumor-specific enzyme can convert a pharmacologically inactive prodrug to the corresponding active parent drug in the vicinity of the tumor. Via this concept a high concentration of toxic anticancer agent can be generated at the tumor site. All tumor cells may be killed if the dose is sufficiently high, which 20 may decrease development of drug resistant tumor cells.

There exist several enzymes that are present at elevated levels in certain tumor tissues. One example is the enzyme β -glucuronidase, which is liberated from certain necrotic tumor areas. Furthermore, several proteolytic enzymes have been shown to be associated with tumor 25 invasion and metastasis. Several proteases, like for example the cathepsins and proteases from

the urokinase-type plasminogen activator (u-PA) system are all involved in tumor metastasis. The serine protease plasmin plays a key role in tumor invasion and metastasis. The proteolytically active form of plasmin is formed from its inactive pro-enzyme form plasminogen by u-PA. The tumor-associated presence of plasmin can be exploited for targeting 5 of plasmin-cleavable prodrugs.

In this invention a new technology is disclosed that can be applied to prepare improved prodrugs or conjugates for targeting drugs to disease-related or organ-specific tissue or cells, for example tumor-specific prodrugs. This technology can furthermore find application in 10 (non-specific) controlled release of compounds, with the aim of facilitating release of the parent moiety. The present invention is deemed to be applicable to all drugs that need to be delivered at a specific target site where a specific disease-related biomolecule can convert the prodrug into the drug or induce conversion of the prodrug into the drug.

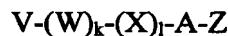
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DESCRIPTION OF THE INVENTION

The technology of this invention relates to novel linker systems to be inserted between a specifier (= part of prodrug to be cleaved by the enzyme) and parent drug. A great number of anticancer prodrugs that have been developed in the past contain a self-eliminating connector or linker, also called self-elimination spacer. This spacer is incorporated between the specifier 20 and the drug in order to facilitate enzymatic cleavage and so enhance the kinetics of drug release (as shown in figure 1). The specifier (which for example can be an oligopeptide substrate for a protease or for example a β -glucuronide substrate for β -glucuronidase) must be site-specifically removed, followed by a spontaneous spacer elimination to release the cytotoxic parent drug. In this invention, greatly improved linker systems are disclosed. These 25 are applicable in prodrugs, for example anticancer prodrugs, and significantly enhance enzymatic activation rates.

More specifically, the invention relates to compounds of the formula:

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wherein:

V is an enzymatically removable specifier,

(W)_k-(X)_l-A is an elongated self-eliminating spacer system,

W and X are each a 1,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula (Y)_m, wherein

Y is a 1,(4+2n) electronic cascade spacer,

5 or a group of formula U, being a cyclisation elimination spacer,

Z is a therapeutic or diagnostic moiety,

k, l and m are independently an integer of 0 (included) to

5 (included),

n is an integer of 0 (included) to 10 (included),

10 with the provisos that:

- when A is (Y)_m: then k+l+m ≥ 1, and
 - if k+l+m=1, then n > 1;
- when A is U: then k+l ≥ 1.

15 These novel elongated linker systems show improved enzymatic activation characteristics, which is demonstrated in the following Examples.

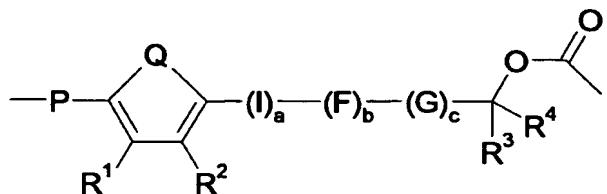
An activatable prodrug according to this invention comprises a specifier V, which is meant to consist of a group that can be site specifically removed and that is covalently attached to a therapeutic or diagnostic moiety Z via the novel elongated self-eliminating connector system

20 (W)_k-(X)_l-A of the invention (figure 2). These self-eliminating connector systems possess increased lengths, which places the parent moiety Z at an increased distance from the specifier.

It is observed that spacers which self-eliminate through a 1,(4+2n)-elimination (n = 0,1,2,3,4,5...10) (for example 1,6-elimination, 1,8-elimination, or 1,10-elimination) are from

25 now called 'electronic cascade' spacers.

According to a preferred embodiment of the invention are the electronic cascade spacers W, X, and Y independently selected from compounds having the formula:

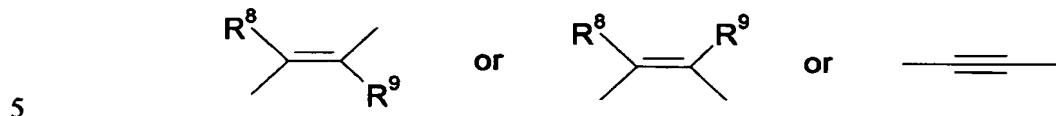


Q = -R⁵C=CR⁶-, S, O, NR⁵, -R⁵C=N-, or -N=CR⁵-
P = NR⁷, O, S

wherein

a, b, and c are independently an integer of 0 (included) to 5 (included);

I, F and G are independently selected from compounds having the formula:



wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, and R⁹ independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphoxy (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphixy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonooxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are independently selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, two or more of the substituents R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, or R⁹ optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

It is further observed that the principle of 1,6-elimination, as such developed in 1981, can be considered one of the most versatile self-elimination principles that can be used in prodrug design. According to this principle, spacer elimination proceeds via the mechanism depicted in figure 3. This particular elimination process has proven to be very successful when applied in the prodrug concept. Spacers that self-eliminate through an electronic cascade sequence as indicated in figure 3 generally show much faster half-lives of elimination than do spacers that eliminate via a cyclisation reaction. This is a significant difference between cyclisation spacers

and electronic cascade spacers.

In the following Examples, para-aminobenzyloxycarbonyl (PABC) and related electronic cascade spacer systems are used because they eliminate more rapidly upon unmasking of the amine, when compared to hydroxybenzyl-based electronic cascade spacers, which need 5 electron-withdrawing substituents on the phenyl part of the spacer in order to let spacer elimination take place. Drug release will not take place when the spacer is an un-substituted hydroxybenzyl electronic cascade spacer.

Most efforts were, in the past, directed to the synthesis of electronic cascade spacers containing electron-withdrawing substituent(s). It was hypothesized that the withdrawal of 10 electrons from the site where enzymatic activation occurs would enhance the rate of enzymatic activation. However, the activation rate of prodrugs containing an electron-withdrawing group on the spacer is usually not significantly different from that of un-substituted electronic cascade spacer containing prodrugs: A chloro-substituent on an aminobenzyl spacer for example only marginally enhances the rate of enzymatic prodrug activation by plasmin. In the 15 case of aminobenzyl spacer-containing anthracycline prodrugs for activation by β -glucuronidase, chloro- or bromo-substituents showed only a marginal effect. It must further be considered that, although electron-withdrawing substituents on aminobenzyl spacers may increase enzymatic activation rates, spacer elimination rates will decrease as a consequence of substituents with such electronic properties. In the case of generation of hydroxylamino benzyl 20 electronic cascade spacers it appeared that indeed electron-donating substituents on the benzyl ring accelerated fragmentation. This effect can probably be ascribed to stabilization of the developing positive charge on the benzylic carbon by these substituents. In some cases, when one or more of the spacer substituents are too electron-withdrawing, spacer elimination will not occur at all. An aminobenzyl spacer containing a nitro substituent at the meta position with 25 respect to the specifier did not self-eliminate to release the free drug. It was also found that a hydroxylamino benzyl spacer with a nitro substituent at the meta position with respect to the specifier showed the slowest spacer elimination rate of such substituted hydroxylamino benzyl spacers. It appears that electron-withdrawing properties of spacer substituents have only marginal impact on enzymatic activation rates, whereas spacer elimination is greatly dependent 30 on electronic properties of spacer substituents and occurs only in a relatively narrow characteristic electronic profile depending on the type of cascade spacer that is used.

In several previously reported prodrugs, containing one electronic cascade spacer, differences

in enzymatic activation rates can still be observed when different parent drugs are connected with the same promoiety or when a parent drug is connected to the same promoiety via a different site of the drug. For example, β -glucuronidase cleaves the glucuronide from a β -glucuronide-cyclisation spacer promoiety much slower when paclitaxel is the parent drug in comparison with the prodrug containing doxorubicin as the parent drug. In another example, a dipeptide derivative of paclitaxel, linked via an aminobenzyl spacer was more readily cleaved by cathepsin B when paclitaxel was linked via its 7-position than via its 2'-position. In addition, half-lives of cathepsin B cleavage of electronic cascade spacer containing prodrugs of doxorubicin or mitomycin C were much shorter than the half-life of the corresponding prodrugs with paclitaxel as the parent drug. Finally, plasmin cleaves the tripeptide from an electronic cascade spacer containing doxorubicin prodrug much more readily than the tripeptide from the corresponding paclitaxel prodrug. Thus, in several prodrug systems the parent drug still exerts a significant effect on the rate of enzymatic activation, even though the mentioned prodrugs all contained one electronic cascade spacer.

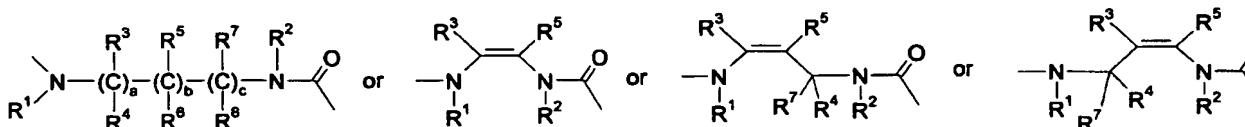
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The invention obviates the above-mentioned drawbacks by reduction of the influence of substituents of the spacer group on prodrug activation and/or spacer elimination and of the parent drug on the rate of enzymatic activation of the prodrug due to the presence of elongated spacer systems.

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The invention is in a second aspect related to compounds of the above-mentioned formula wherein group U is a cyclisation spacer, from now called ‘ ω -amino aminocarbonyl’ cyclisation spacer, and Z is a molecule having a hydroxyl group.

25 More preferably, the ω -amino aminocarbonyl cyclisation elimination spacer U of the invention is a compound having the formula:



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wherein:

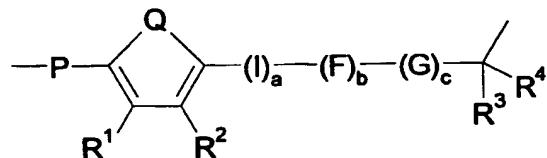
a is an integer of 0 or 1; and

b is an integer of 0 or 1; and

c is an integer of 0 or 1; provided that

a + b + c = 2 or 3;

- 5 and wherein R¹ and/or R² independently represent H, C₁₋₆ alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), disubstituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), 10 carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group; and
- R³, R⁴, R⁵, R⁶, R⁷, and R⁸ independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl,
- 15 C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), disubstituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, two or 20 more of the substituents R¹, R², R³, R⁴, R⁵, R⁶, R⁷, or R⁸ optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.
- 25 In a third aspect, the invention relates to compounds of the above-mentioned formula wherein spacer group A is an electronic cascade spacer having the formula:



Q = -R⁵C=CR⁶-, S, O, NR⁵, -R⁵C=N-, or -N=CR⁵-
 P = NR⁷, O, S

wherein

a, b, and c are independently an integer of 0 to 5;

I, F and G are independently selected from compounds having the formula:



wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, and R⁹ independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), disubstituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me,
 10 CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where
 15 R_x, R_x¹ and R_x² are independently selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, two or more of the substituents R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, or R⁹ optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

In one embodiment the elongated spacer system (W)_k- (X) _l- A is a molecule that self-eliminates via a $1,(4+2n)$ -elimination ($n = 2,3,4,5\dots 10$), for example a 1,8-elimination (figure 4). The length of this novel spacer system can be extended, for example to a 1,10-elimination spacer system, in which two or more double or triple bonds instead of one are conjugated with the aromatic part of the spacer (figure 5).

In another embodiment, the spacer system of the invention consists of two or more electronic
25 cascade spacers that are connected to one another. Release of the leaving group (the drug)
occurs after two or more subsequent spacer eliminations.

In again another embodiment, prodrugs of hydroxyl functionality containing drugs (such as for example paclitaxel) are claimed that contain both one or more electronic cascade spacers and a cyclisation spacer.

30 In a preferred embodiment the spacer that is directly connected to the paclitaxel molecule is an ω -amino aminocarbonyl cyclisation spacer that is linked to the 2'-position of paclitaxel via a

carbamate linkage. A convenient synthetic route to this class of paclitaxel derivatives is disclosed.

In another embodiment the elongated electronic cascade spacer system is coupled to the phenolic hydroxyl group of the drug moiety via an ether linkage. When the leaving group (i.e., 5 the drug) is an phenolic hydroxyl group, a para-aminobenzylether has been reported to self-eliminate.

The elongated spacer systems provide for improved enzymatic activation characteristics.

The self-eliminating connector systems in this invention possess increased lengths with respect 10 to an electronic cascade spacer system available at present. It is observed that one end of the linker system must be able to react with the specifier, for example the tripeptide that is a substrate for plasmin. Typically, this end of the spacer system is an amino group or a hydroxyl group, but it can also be another functionality. The functionality at the other end of the linker system must be able to react with the drug. Typically, this end of the spacer system is a 15 hydroxyl group, but it can also be another functionality. In one embodiment this functionality reacts with an amino group of the drug to form a carbamate linkage between linker and drug. In another embodiment, this functionality reacts with a hydroxyl group of the drug to form a carbonate linkage between linker and drug. In again another embodiment, this functionality reacts with a sulphydryl group of the drug to form a thiocarbonate linkage between linker and 20 drug. In again another embodiment this functionality reacts with a carboxylic acid group of the drug to form an ester linkage between linker and drug.

Typically, the spacer system is p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl, p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl, p-amino-25 cinnamylloxycarbonyl, p-aminocinnamylloxycarbonyl-p-aminobenzylloxycarbonyl, p-amino-benzylloxycarbonyl-p-aminocinnamylloxycarbonyl, p-aminocinnamylloxycarbonyl-p-amino-cinnamylloxycarbonyl, p-aminophenylpentadienyloxycarbonyl, p-aminophenylpenta-30 dienyloxycarbonyl-p-aminocinnamylloxycarbonyl, p-aminophenylpentadienyloxycarbonyl-p-aminobenzylloxycarbonyl, p-aminophenylpentadienyloxycarbonyl-p-aminophenylpenta-dieneoxycarbonyl, p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminocinnamylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminobenzyl-oxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-amino-cinnamylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminobenzylloxycarbonyl-p-aminocinnamylloxycarbonyl(methylamino)ethyl(methylamino)-

carbonyl, p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl(methylamino)ethyl-(methylamino)carbonyl, p-aminobenzylloxycarbonyl-p-aminobenzyl, p-aminobenzyl-oxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzyl, p-aminocinnamyl, p-aminocinnamylloxycarbonyl-p-aminobenzyl, p-aminobenzylloxycarbonyl-p-aminocinnamyl, p-aminocinnamylloxycarbonyl-p-aminocinnamyl, p-aminocinnamyl, p-aminophenylpentadienyl, p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl, p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl, or p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

- In the compounds of formula V-(W)_k-(X)_l-A-Z, the specifier V typically contains a substrate molecule that is specifically cleaved by an enzyme present in the vicinity of the target cells, for example tumor cells. More preferably, the specifier V contains a substrate that is specifically cleaved by an enzyme present at elevated levels in the vicinity of the target cells as compared to other parts of the body, and most preferably the enzyme is present only in the vicinity of the target cells.
- The specifier V may also contain a moiety that targets the compounds of formula V-(W)_k-(X)_l-A-Z to the target site by selective complexing with a receptor or other receptive moiety associated with a given target cell population or by causing accumulation of compounds V-(W)_k-(X)_l-A-Z in the vicinity of the target cells by another mechanism. This targeting moiety may, for example, be bombesin, transferrin, gastrin, gastrin-releasing peptide, a molecule that specifically binds $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ -integrin receptors, such as RGD-containing peptides, platelet-derived growth factor, IL-2, IL-6, a tumor growth factor, vaccinia growth factor, insulin and insulin-like growth factors I en II, an antigen-recognizing immunoglobulin or an antigen-recognizing fragment thereof, or a carbohydrate. Preferably, that antigen recognized by the immunoglobulin (or fragment thereof) is specific for the target cells, e.g. a tumor-specific antigen. The specifier V may also contain a polymer, which causes accumulation of compounds V-(W)_k-(X)_l-A-Z in the vicinity of the target cells, e.g. tumor cells, because of the Enhanced Permeability and Retention (EPR) effect.

- In one embodiment, the specifier is a di-, tri-, or oligopeptide which consists of an amino acid sequence specifically recognized by a protease, for example plasmin, a cathepsin, cathepsin B, prostate-specific antigen (PSA), urokinase-type plasminogen activator (u-PA), or a member of the family of matrix metalloproteinases, present in the vicinity of the target cells, for example tumor cells, or, in another embodiment, a β -glucuronide that is specifically recognized by β -

glucuronidase present in the vicinity of tumor cells. In again another embodiment the specifier is a nitro-aromatic moiety that can be reduced under hypoxic conditions or by nitroreductases. After removal of the nitro-aromatic specifier, elimination of the spacer systems described in this invention leads to drug release. It can be understood that any specifier that is specifically cleaved following recognition by a disease-specific and/or organ-specific enzyme and/or receptor can be incorporated into prodrugs that contain the linker systems claimed in this invention.

The moiety Z is a therapeutic or diagnostic moiety. Z can for instance be an anticancer drug, an antibiotic, an anti-inflammatory agent, or an anti-viral agent. Typically, the moiety Z is an anticancer drug. Preferably the anticancer drug is the amino containing daunorubicin, doxorubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, mitomycin C, mitomycin A, 9-amino camptothecin, aminopterin, actinomycin, bleomycin, N⁸-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, tallysomycin, or derivatives thereof. The anticancer drug can also be the hydroxyl containing etoposide, camptothecin, irinotecan, topotecan, 9-amino camptothecin, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxybicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, doxorubicin, morpholine-doxorubicin, N-(5,5-diacetoxypentyl) doxorubicin, vincristine, vinblastine, or derivatives thereof. The anticancer drug can also be the sulphydryl containing esperamicin, 6-mercaptopurine, or derivatives thereof. The drug can also be the carboxyl containing methotrexate, camptothecin (ring-opened form of the lactone), butyric acid, retinoic acid, or derivatives thereof.

To show the principle of elimination of elongated spacer systems, tumor-specific prodrugs that are selectively hydrolyzed by the tumor-associated protease plasmin were synthesized. The synthesized prodrugs consist of a tripeptide specifier that is coupled to the drug via an elongated self-eliminating spacer. The tripeptide specifier contains an amino acid sequence that is specifically recognized by the tumor-associated enzyme plasmin. The synthesis of these derivatives is disclosed.

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There is an increasing body of literature that links production of certain proteases to tumor malignancy. Mostly, proteolytic activity is required for tumor cells when they become invasive and form metastases. A primary tumor is encapsulated in an extracellular matrix, which

consists of proteins. In order to form metastases, the primary tumor must break through this matrix. For this reason, enhanced expression of proteolytic enzymes by invading and metastasizing tumors is generated. Recent studies indicate that proteases are involved also in earlier stages of tumor progression, at both primary and metastatic sites. A number of 5 proteases, like cathepsins, the u-PA system, and the matrix metalloproteinases, take part in the proteolytic cascade.

The u-PA system has received broad attention in the literature, especially in the last decade. Several invasive and metastasizing human tumors express a significantly higher plasminogen activator activity in comparison with normal tissue. An increased activity and expression of u-10 PA is found in several tumor cell lines and human solid tumors, like lung tumors, prostate cancers, breast cancers, ovarian carcinomas and several other cancer types. u-PA is an important enzyme in proteolytic reactions that are required for the spreading and invasiveness of cells, both in cancer and in tissue remodeling processes. u-PA interacts with a specific high-affinity receptor on the cell surface. Receptor-bound u-PA is catalytically active on the surface 15 of the cell without requiring internalization. It interacts with plasminogen to produce plasmin that is still bound to the cell surface. The high u-PA level via this pathway leads to elevated levels of plasmin. There exists substantial evidence that the protease plasmin itself plays a key role in tumor invasion and metastasis. Plasmin itself catalyses the breakdown of extracellular matrix proteins. Thus, the plasminogen activator system is intimately associated with tumor 20 metastasis. Even the process of angiogenesis, nowadays considered as an important target mechanism for the development of new therapeutic strategies, is a urokinase dependent process. The plasminogen activation system may be involved in cell adhesion processes by regulating integrin functions. Vascular endothelial growth factor (VEGF), an angiogenic molecule, is suggested to interact with u-PA in tumor progression. u-PA catalyzed plasmin 25 generation proved to be an important determinant of tumor metastasis in many experiments with animal model systems.

For the reasons outlined above, plasmin can be a very promising enzyme for the targeting of peptide prodrugs of anticancer agents. Active plasmin is localized in tumor tissue because it is formed from its inactive pro-enzyme form plasminogen by u-PA, produced by cancer and/or 30 stroma cells. In the blood circulation active plasmin is rapidly inhibited by inhibitors that block the active site, such as α_2 -antiplasmin. Cell-bound plasmin as present in tumor tissue is not inhibited. In addition, plasmin is suitable as a target enzyme for prodrugs, because it is generated at the end of the proteolytic cascade. One molecule of u-PA can generate more than

one molecule of plasmin.

The amino acid sequence of the tripeptide to be a plasmin substrate must be chosen such that it is a specific substrate for the serine protease plasmin. The C-terminal amino acid that is 5 coupled to the spacer-drug moiety is arginine or lysine, preferably an *L*-lysine residue. Plasmin is known to cleave most easily after a lysine residue. The amino acid at the N-terminus possesses the *D*-configuration in order to prevent *in vivo* cleavage by ubiquitous amino peptidases. Protecting the N-terminal amino function by a Boc or Fmoc group can also prevent unwanted peptidase cleavage. The amino acid in the middle is preferably a hydrophobic *L*- 10 amino acid and is selected from the group consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, cyclohexylglycine, tryptophan and proline. Preferred tripeptide sequences are D-alanylphenylalanyllysine, D-valylleucyllysine, D-alanylleucyllysine, D-valylphenylalanyllysine, D-valyltryptophanyllysine, and D-alanyltryptophanyllysine.
By converting the two amino groups of the tripeptide into the corresponding ammonium salts, 15 the water solubility of the prodrug should be improved.

In the present invention the synthesis and application of new elongated spacer systems is described. In one embodiment, this spacer self-eliminates through a 1,(4+2n)-elimination process (figures 4,5). These 1,(4+2n)-elimination spacers are elongated with respect to the 20 conventional 1,6-elimination spacer. Proof of principle of 1,8-elimination was delivered upon chemical reduction of the nitrocinnamyl carbonate derivative of paclitaxel using zinc and acetic acid (figures 6,7). Released paclitaxel was isolated in good yield. Firstly, the doubly protected tripeptide was synthesized (figure 8). The 1,8-elimination spacer itself was synthesized from 4-nitrocinnamyl alcohol as depicted in figure 9. 4-Aminocinnamyl alcohol 25 was incorporated between doxorubicin and a tripeptide for plasmin activation (figure 10).
What is also disclosed in this invention are prodrugs that contain two or more electronic cascade spacers connected to one another, incorporated between specifier and drug (figure 11). Prodrugs containing linker systems of this kind have not been reported before. This embodiment of the present invention was exemplified by synthesizing two prodrugs containing 30 a tripeptide specifier coupled to doxorubicin or paclitaxel via two 1,6-elimination spacers. This protected tripeptide was subsequently coupled with 4-aminobenzyl alcohol, and the resulting benzylic alcohol was activated with 4-nitrophenyl chloroformate to yield the corresponding 4-nitrophenyl carbonate (figure 12). In a very efficient reaction a second molecule of 4-

aminobenzyl alcohol was coupled to the activated carbonate in which hydroxy benzotriazole (HOBt) was employed as a catalyst to yield the corresponding tripeptide-double spacer conjugate (figure 13). When this reaction was performed using diphenyl phosphinic acid as a catalyst, the product was isolated in only 16 percent yield (figure 14). The peptide-double 5 spacer conjugate was incorporated into a doxorubicin prodrug (figure 15) and a paclitaxel prodrug (figure 16), by subsequent chloroformate activation, coupling with the drug and final deprotection. A double spacer-containing doxorubicin prodrug with a tryptophan residue instead of phenylalanine was also synthesized (figures 17,18). According to a further embodiment a third 4-aminobenzyl alcohol spacer was reacted with the 4-nitrophenyl 10 carbonate activated tripeptide-double spacer conjugate to yield the corresponding tripeptide-triple spacer conjugate (figure 19). This compound was subsequently converted to the corresponding triple electronic cascade spacer containing doxorubicin prodrug employing a similar route as depicted in figures 15 and 16.

What is also claimed are prodrugs in which the promoiety is coupled to a hydroxyl group of 15 parent moiety Z via a carbamate linkage. These carbamate coupled prodrugs contain an elongated linker system that contains both one or more electronic cascade spacers and an ω -amino aminocarbonyl cyclisation spacer (figure 20). Paclitaxel-2'-carbamate prodrugs of this type were synthesized via a novel convergent route, which leads to high yields, as claimed in claims 35 and 36. Paclitaxel will be released after one or more 1,(4+2n)-eliminations (n = 20 0,1,2,3,4,5,...10) and a subsequent intramolecular cyclisation. In the present invention the cyclisation spacer is connected to the 2'-OH group of paclitaxel through a carbamate linkage (figure 20). Firstly, paclitaxel was selectively activated at the 2'-position (figure 21) Secondly, a mono-protected cyclisation spacer was coupled to the 2'-activated paclitaxel analog and the protective group was removed under acidic conditions to yield the first fragment (figure 22). 25 The second fragment was synthesized by connecting the 1,6-elimination spacer to the tripeptide specifier and subsequent 4-nitrophenyl chloroformate activation of the benzylic alcohol function (figure 12). Then, both fragments were coupled to one another (figure 23) and the coupled product was deprotected (figure 24). Coupling of two separate fragments according to this strategy in which in the final stage the chemical link between the two spacers 30 is established, did provide the most efficient route to the paclitaxel prodrug. This is a novel route to obtain prodrugs of this type, in which a specifier is connected to a hydroxyl containing drug via an electronic cascade spacer system (connected to the specifier) and a cyclisation spacer (connected to the drug). The preparation of two other prodrugs of paclitaxel that contain

both an electronic cascade spacer system and a cyclisation spacer is depicted in figures 25 – 27. In figure 28 previously reported plasmin-activatable prodrugs containing one electronic cascade spacer are depicted.

- 5 In a further aspect the invention relates to processes for the synthesis of the prodrugs as defined above. The invention e.g. relates to a process for the synthesis of prodrugs as defined above having at least one electronic cascade spacer group, and an ω -amino aminocarbonyl cyclisation elimination spacer group, connected to each other, incorporated between a specifier group and a drug molecule such that said drug molecule is connected to said cyclisation 10 elimination spacer group, via the hydroxyl functionality of the drug molecule, by coupling a first electronic cascade spacer group, connected to said specifier group, if desired via at least one, second electronic cascade spacer group, being the same or different as said first electronic cascade spacer group, to said cyclisation elimination spacer group. In a preferred process said drug molecule is paclitaxel, and in a first step a cyclisation elimination spacer group is coupled 15 to paclitaxel via its 2'-hydroxyl group through a carbamate linkage via addition of the free spacer-amine to a 4-nitrophenyl carbonate activated drug, followed by deprotection to obtain a first fragment consisting of a cyclisation spacer connected with paclitaxel, and in a second step one or more 1,(4+2n) electronic cascade spacers, being the same or different, wherein n is an integer of 0 to 10, are coupled to a specifier group, subsequently activated to the corresponding 20 4-nitrophenyl carbonate, whereafter in a third step the fragments obtained in the first and second step are coupled to one another under basic reaction conditions.

The invention further relates to a process for the synthesis of prodrugs as defined above, in which electronic cascade spacers are connected to one another by coupling of the terminal alcohol group of an electronic cascade spacer to the aniline amino group of another electronic 25 cascade spacer through a carbamate linkage by conversion of the alcohol group of the first-mentioned electronic cascade spacer to the corresponding 4-nitrophenyl carbonate and reacting this molecule with the other electronic cascade spacer in the presence of a catalytic amount of 1-hydroxybenzotriazole either in the presence or absence of base.

- 30 In yet another aspect the invention relates to the use of any of the compounds defined above for the manufacture of a pharmaceutical preparation for the treatment of a mammal being in need thereof. The invention also relates to methods of treating a mammal being in need

thereof, whereby the method comprises the administration of a pharmaceutical composition to the mammal in a therapeutically effective dose.

In a further aspect the invention relates to a process for preparing a pharmaceutical composition containing a compound as defined above, to provide a solid or a liquid formulation for administration orally, topically or by injection. Such a process at least comprises the step of mixing the compound with a pharmaceutically acceptable carrier.

The invention also relates to pharmaceutical compositions comprising the compounds of the invention as defined above. The compounds of the invention may be administered in purified form together with a pharmaceutical carrier as a pharmaceutical composition. The preferred form depends on the intended mode of administration and therapeutic or diagnostic application. The pharmaceutical carrier can be any compatible, nontoxic substance suitable to deliver the compounds of the invention to the patient. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as (sterile) water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters, alcohol, fats, waxes, and inert solids may be used as the carrier. A pharmaceutically acceptable carrier may further contain physiologically acceptable compounds that act, e.g. to stabilise or to increase the absorption of the compounds of the invention. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilisers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. Pharmaceutically acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions.

For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that may be

added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous 5 release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain colouring and flavouring to increase patient acceptance.

The compounds of the invention are however preferably administered parentally. Preparations 10 of the compounds of the invention for parental administration must be sterile. Sterilisation is readily accomplished by filtration through sterile filtration membranes, optionally prior to or following lyophilisation and reconstitution. The parental route for administration of compounds of the invention is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intramuscular, intraarterial or intralesional routes. The 15 compounds of the invention may be administered continuously by infusion or by bolus injection. A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile 0.9% NaCl or 5% glucose optionally supplemented with a 20% albumin solution and 1 mg to 10 g of the compound of the invention, depending on the particular type of compound of the invention and its required dosing regime. Methods for preparing 20 parenterally administrable compositions are well known in the art and described in more detail in various sources, including, for example, Remington's Pharmaceutical Science (15th ed., Mack Publishing, Easton, PA, 1980) (incorporated by reference in its entirety for all purposes).

The invention also relates to compounds as defined above, wherein the specifier V is removed 25 by an enzyme that is transported to the vicinity of target cells or target tissue via antibody-directed enzyme prodrug therapy (ADEPT), polymer-directed enzyme prodrug therapy (PDEPT), virus-directed enzyme prodrug therapy (VDEPT) or gene-directed enzyme prodrug therapy (GDEPT) (see e.g. U.S. Pat. No. 4,975,278, Melton et al., 1996, J. Natl. Can. Inst. 88(3/4):153-165).

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The invention is further exemplified by the following Examples. These examples are for illustrative purposes and are not intended to limit the scope of the invention.

BRIEF DESCRIPTION OF DRAWINGS

- Figure 1 shows schematically the conversion of a spacer containing tripartate prodrug into the parent drug.
- Figure 2 shows schematically the structure of an elongated spacer containing prodrug.
- 5 Figure 3 shows the principle of 1,6-elimination.
- Figure 4 shows the principle of 1,8-elimination.
- Figure 5 shows the principle of 1,10-elimination.
- Figure 6 shows the synthesis of the model paclitaxel-containing compound to prove the principle of 1,8-elimination.
- 10 Figure 7 shows the mechanism for the release of paclitaxel after reduction and 1,8-elimination.
- Figure 8 shows the synthesis of the doubly Aloc-protected D-Ala-Phe-Lys tripeptide.
- Figure 9 shows the synthesis of the 1,8-elimination spacer para-aminocinnamyl alcohol (PACA).
- Figure 10 shows the synthesis of a 1,8-elimination spacer containing prodrug.
- 15 Figure 11 shows schematically the structure of a prodrug containing two or more electronic cascade spacers.
- Figure 12 shows the synthesis of para-nitrophenyl (PNP) carbonate-activated tripeptide-spacer conjugate.
- Figure 13 shows the catalytic coupling of a second electronic cascade spacer molecule (para-aminobenzyl alcohol (PABA)) to the 4-nitrophenyl carbonate-activated tripeptide-spacer conjugate in the presence of hydroxy benzotriazole (HOBt).
- 20 Figure 14 shows a reaction to chemically link two electronic cascade spacer molecules by coupling a second electronic cascade 1,6-elimination spacer molecule to a 4-nitrophenyl carbonate activated tripeptide-1,6-elimination spacer conjugate in the presence of catalytic amounts of diphenyl phosphinic acid.
- Figure 15 shows the synthesis of a doxorubicin containing double 1,6-elimination spacer containing prodrug.
- 25 Figure 16 shows the synthesis of a paclitaxel containing double 1,6-elimination spacer containing prodrug.
- Figure 17 shows the synthesis of a tryptophan-containing tripeptide double spacer conjugate.
- Figure 18 shows the synthesis of a tryptophan-containing doxorubicin prodrug.
- 30 Figure 19 shows the synthesis of a doxorubicin containing triple 1,6-elimination spacer containing prodrug.

Figure 20 shows schematically the structure of a prodrug of paclitaxel that contains both an electronic cascade spacer and a cyclisation spacer coupled to the drug via a 2'-carbamate linkage.

Figure 21 shows the regioselective synthesis of 2'-(4-nitrophenyl carbonate) activated paclitaxel using 4-nitrophenyl chloroformate at low temperature.

Figure 22 shows the synthesis of the acid protected paclitaxel- ω -amino aminocarbonyl cyclisation spacer conjugate.

Figure 23 shows the coupling of the acid protected paclitaxel- ω -amino aminocarbonyl cyclisation spacer conjugate to the 4-nitrophenyl carbonate activated tripeptide-1,6-elimination spacer conjugate.

Figure 24 shows the deprotection reaction to obtain the paclitaxel prodrug that contains a 1,6-elimination spacer and an ω -amino aminocarbonyl cyclisation spacer.

Figure 25 shows the preparation of a paclitaxel prodrug that contains two 1,6-elimination spacers and an ω -amino aminocarbonyl cyclisation spacer.

Figure 26 shows the synthesis of para-nitrophenyl (PNP) carbonate-activated doubly para-nitrobenzyloxycarbonyl-protected tripeptide-spacer conjugate.

Figure 27 shows the preparation of a paclitaxel prodrug that contains a 1,8-elimination spacer and an ω -amino aminocarbonyl cyclisation spacer.

Figure 28 shows the structure of previously reported doxorubicin and paclitaxel prodrugs containing one electronic cascade spacer.

EXAMPLESExample 15 Synthesis of 2'-[4-nitrocinnamyl carbonate]-paclitaxel 1.

To a solution of 200 mg (1.12 mmol, 4.8 equiv) 4-nitrocinnamyl alcohol in dry dichloromethane/tetrahydrofuran under an Argon atmosphere was added pyridine (94 µl, 5.0 equiv) and 4-nitrophenyl chloroformate (236 mg, 5.0 equiv). The reaction mixture was stirred 10 for 12 h at room temperature. The mixture was cooled to 0 °C and a catalytic amount of DMAP, a few drops of triethyl amine and 200 mg paclitaxel (1.0 equiv) were added. The reaction mixture was stirred at room temperature for 12 h. Solvents were evaporated and the remaining solid was dissolved in dichloromethane. The organic layer was thoroughly washed 15 with a saturated sodium bicarbonate solution, 0.5 N potassium bisulfate and brine and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow oil was purified by means of column chromatography (ethyl acetate – hexane; 1:1), to yield 144 mg of 1 (58%). M.P. 151 °C; ¹H-NMR (300 MHz, CDCl₃) δ 1.17 (s, 3H, 17), 1.22 (s, 3H, 16), 1.70 (s, 3H, 19), 1.96 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.46 (s, 3H, 4-OAc), 2.55 (m, 1H, 6a), 3.82 (d, 1H, J=7.0 Hz, 3), 4.26 (d, 1H, J=8.4 Hz, 20b), 4.32 (d, 1H, J=8.4 Hz, 20a), 4.39 (m, 1H, 7), 20 4.87 (bt, 2H, CH₂-spacer), 4.99 (bd, 1H, J=7.9 Hz, 5), 5.46 (d, 1H, J=2.8 Hz, 2'), 5.72 (d, 1H, J=7.1 Hz, 2), 6.01 (m, 1H, 3'), 6.26 (bt, 1H, 13), 6.34 (s, 1H, 10), 6.43 (dt, 1H, J=16.0 Hz, HC=CH-CH₂), 6.75 (d, 1H, J=16.0 Hz, HC=CH-CH₂), 7.35-7.67 (m, 13H, aromatic), 7.75 (d, 2H, J=7.2 Hz, aromatic), 8.15 (d, 2H, J=7.2 Hz, aromatic), 8.19 (d, 2H, J=8.7 Hz, nitrophenyl) ppm; MS (FAB) *m/e* 1059 (M + H)⁺, 1081 (M + Na)⁺; Anal. (C₅₇H₅₈N₂O₁₈·2½H₂O) calculated 25 C 62.01%, H 5.75%, N 2.54%, measured C 62.06%, H 5.31%, N 2.60%.

Example 2Principle of 1,8-elimination: chemical reduction of the nitrocinnamyl carbonate 1.

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36 mg of 2'-[4-nitrocinnamyl carbonate]-paclitaxel 1 was dissolved in 8 ml methanol and 2 ml acetic acid. A catalytic amount of zinc powder was added and the red mixture was stirred for 12 h. Dichloromethane was added and the organic layer was washed with saturated sodium

bicarbonate, 0.5 N potassium bisulfate, brine, and water and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (ethyl acetate – hexane; 2:1), to yield 28 mg of paclitaxel (confirmation by 300 MHz $^1\text{H-NMR}$) and 4 mg of unreacted starting compound. When the
5 compound was stirred in the absence of zinc powder under the same conditions, no paclitaxel was formed, indicating that reduction of the nitro group by zinc leads to the release of paclitaxel.

Example 3

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Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-OH 9.

Step a: Synthesis of Fmoc-Phe-Lys(Boc)-OBu 4.

15 To a solution of 2.50 g Fmoc-Phe-ONSu 2 (ONSu = N-hydroxysuccinimide) (5.16 mmol) in dry dichloromethane under an Argon atmosphere were added at 0 °C 0.791 ml triethyl amine (1.1 eq.) and 1.92 g H-Lys(Boc)-OBu-HCl 3 (1.1 eq.). The reaction mixture was stirred at room temperature for 5 hours, then dichloromethane was added and the organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water. The organic layer was
20 dried over anhydrous sodium sulphate and evaporated. The resulting white solid 4 (3.08 g, 89%) was used without further purification. M.P. 93 °C; $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.10-1.90 (m, 24H, 6 CH_2 -Lys and 18 tert-butyl), 3.06 (m, 2H, N- CH_2 -Lys and benzylic), 4.19 (t, 1H, Fmoc), 4.25-4.55 (m, 4H, 2 Fmoc and 2 H_α), 7.19-7.78 (m, 13H, aromatic) ppm; MS (FAB) m/e 672 ($M + H$) $^+$, 694 ($M + \text{Na}$) $^+$; $\text{C}_{39}\text{H}_{49}\text{N}_3\text{O}_7$ calculated C 69.72%, H 7.35%, N
25 6.25%, measured C 69.69%, H 7.48%, N 6.22%.

Step b: Synthesis of Boc-D-Ala-Phe-Lys(Boc)-OBu 7.

3.08 g (4.58 mmol) of Fmoc-Phe-Lys(Boc)-OBu 4 was dissolved in 100 ml of
30 dioxane/methanol/2N sodium hydroxide (70/25/5) and stirred at room temperature for approximately 1 hour. The reaction mixture was neutralised with acetic acid (0.571 ml) and organic solvents were evaporated. Water and dioxane was added and the solution was freeze dried. Diisopropylether was added to the resulting solid. After filtration, the filtrate was

evaporated. The residual product **5** was dissolved in dry dichloromethane and added at 0 °C to a solution of 1.19 g (4.16 mmol) Boc-D-Ala-ONSu **6** and 0.634 ml (1.1 eq.) of triethyl amine in dry dichloromethane. The reaction mixture was stirred overnight after which dichloromethane was added. The organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulphate and evaporated. The product was purified by means of column chromatography (SiO₂ – CHCl₃/MeOH 20/1) to afford 2.56 g (4.13 mmol, 99%) of Boc-D-Ala-Phe-Lys(Boc)-OBu **7** as a white foam. M.P. 59 °C; ¹H-NMR (300 MHz, CDCl₃): δ 1.25 (d, 3H, CH₃-Ala), 1.43 (bs, 27H, tert-butyl), 1.00-1.90 (m, 6H, CH₂-Lys), 2.80-3.30 (m, 4H, N-CH₂-Lys and benzylic), 4.15 (m, 1H, H α), 4.35 (m, 1H, H α), 4.64 (bd, 1H, H α), 7.15-7.35 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 621 (M + H)⁺, 643 (M + Na)⁺; C₃₂H₅₂N₄O₈ ($\cdot\frac{1}{2}$ H₂O) calculated C 61.03%, H 8.48%, N 8.90%, measured C 61.15%, H 8.44%, N 8.66%.

Step c: Synthesis of D-Ala-Phe-Lys-OH **8**.

2.56 g (4.13 mmol) Boc-D-Ala-Phe-Lys(Boc)-OBu **7** was stirred in a solution of HCl in EtOAc (3M). After 5 hours the solvent was evaporated, tert-butanol was added and evaporated twice to remove remaining hydrochloric acid. The resulting product was freeze dried in a mixture of dioxane/water to yield a cream coloured powder **8**, which was used without further purification. ¹H-NMR (300 MHz, D₂O): δ 0.94 (d, 3H, CH₃-Ala), 1.10-1.85 (m, 6H, CH₂-Lys), 2.75-2.84 (m, 3H, N-CH₂-Lys and benzylic), 3.09 (dd, 1H, benzylic), 3.54 (dd, 1H, H α), 3.98 (dd, 1H, H α), 4.54 (q, 1H, H α), 7.10-7.22 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 365 (M + H)⁺.

Step d: Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-OH **9**.

To solution of 706 mg (1.61 mmol) D-Ala-Phe-Lys-OH **8** in water/acetonitrile was added triethyl amine until a pH of 9 – 9.5 was reached. Then a solution of 704 mg (2.2 eq.) Aloc-ONSu in acetonitrile was added and the reaction mixture was kept basic by adding triethyl amine. After the pH of the mixture did not alter anymore, a 0.5 M solution of HCl was added until a pH of 3 was reached. The mixture was thoroughly extracted with dichloromethane. The organic layer was washed with water and the water layer was extracted again with dichloromethane. The organic layer was dried over anhydrous sodium sulphate and evaporated

to dryness to result in the desired product **9** as a cream coloured foam (742 mg, 86%). M.P. 141 °C; $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.10-1.95 (m, 6H, CH_2 -Lys), 1.21 (d, 3H, CH_3 -Ala), 2.90-3.30 (m, 4H, N- CH_2 -Lys and benzylic), 4.20 (m, 1H, $\text{H}\alpha$), 4.55 (m, 5H, $\text{H}\alpha$ and 4 Aloc), 4.76 (bd, 1H, $\text{H}\alpha$), 5.17-5.31 (m, 4H, Aloc), 5.83-5.92 (m, 2H, Aloc), 7.20-7.28 (m, 5H, aromatic) ppm; MS (FAB) m/e 533 ($\text{M} + \text{H}$) $^+$, 555 ($\text{M} + \text{Na}$) $^+$; $\text{C}_{26}\text{H}_{36}\text{N}_4\text{O}_8$ calculated C 58.63%, H 6.81%, N 10.52%, measured C 58.54%, H 6.81%, N 10.28%.

Example 4

10 **Synthesis of 4-aminocinnamyl alcohol **10**.**

To a solution of 1.5 g (8.37 mmol) of 4-nitrocinnamyl alcohol in THF/methanol (60 mL, 1:1 v/v) was added a catalytic amount of Raney Nickel and hydrazine monohydrate (1.22 mL, 25.1 mmol). The mixture was stirred at room temperature for 3 h, additional hydrazine monohydrate (1.22 mL) being added after 1.5 h. The reaction mixture was filtered over HFLO and concentrated under reduced pressure to 5 mL. Dichloromethane (100 mL) was added and the resultant solution was washed with water, dried over Na_2SO_4 , filtered, and concentrated, which gave **10** (1.24 g, 8.31 mmol, 99%).

15 $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 4.24 (d, 2H, $J = 6.1$ Hz, CH_2OH), 6.11 – 6.20 (dt, 1H, $J = 6.1$ Hz, $J = 15.8$ Hz, $\text{CH}=\text{CH}-\text{CH}_2\text{OH}$), 6.48 (d, 1H, $J = 15.8$ Hz, $\text{CH}=\text{CH}-\text{CH}_2$), 6.62 (d, 2H, $J = 11.1$ Hz, aromatic), 7.19 (d, 2H, $J = 11.0$ Hz, aromatic) ppm; MS (EI) m/e 149(M) $^+$.

Example 5

25 **Synthesis of Fmoc-D-Ala-Phe-Lys(Fmoc)-PACA **11**.**

To a solution of D-Ala-Phe-Lys-OH **8** (3.20 g, 8.79 mmol) in a 7:3 mixture of water and acetonitrile (300 mL) was added triethylamine until a pH of 8.5 was reached. A solution of Fmoc-OSu (5.93 g, 17.6 mmol) in acetonitrile (50 ml) was added. The pH of the resultant 30 solution was kept at a pH of 8.5-9.0 by the addition of triethylamine. When the pH did no longer change, a 1 N aqueous HCl solution was added to neutralize the solution. The solution was concentrated under reduced pressure to remove acetonitrile. The resultant aqueous solution was extracted 4 times with ethyl acetate. The combined organic layers were dried over

Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was thoroughly washed with diisopropyl ether to remove apolar contaminations. This gave 5.54 g (6.85 mmol, 78%) of crude **9b**.

A solution of **9b** (500 mg, 0.618 mmol) in THF (50 mL) was cooled to –40 °C. Then, *N*-methylmorpholine (75 µL, 0.68 mmol) and isobutyl chloroformate (89 µL, 0.68 mmol) were added consecutively. The resultant solution was stirred at –40 °C for 3.5 h. A solution of para-aminocinnamyl alcohol (111 mg, 0.742 mmol) in THF (20 mL) was added slowly. The reaction mixture was stirred at –20 °C for 5 h and then concentrated under reduced pressure. The crude product was purified by means of column chromatography (SiO₂ – CH₂Cl₂/MeOH 93/7). This gave 516 mg of **11** (0.549 mmol, 89%).

¹H NMR (300 MHz, CDCl₃/CD₃OD) δ 1.17 (d, 3H, *J* = 6.8 Hz, CH₃ of Ala), 1.20 – 2.00 (m, 6H, 3 x CH₂-Lys), 2.89 – 3.26 (m, 4H, N-CH₂ of Lys and Ph-CH₂ of Phe), 3.88 – 4.58 (m, 11H, CH₂ and CH of Fmoc and CH₂ of spacer and 3 x H α), 6.21 (dt, 1H, *J* = 15.9 Hz, *J* = 5.8 Hz, CH=CH-CH₂), 6.48 (d, 1H, *J* = 15.7 Hz, CH=CH-CH₂) 7.15 – 7.43 (m, 17H, aromatic), 7.52 – 7.57 (m, 2H, aromatic), 7.67 (d, 1H, *J* = 7.4 Hz, aromatic), 7.71 (d, 1H, *J* = 7.4 Hz, aromatic) ppm; MS (FAB) *m/e* 940 (M + H)⁺.

Example 6

20 **Synthesis of Fmoc-D-Ala-Phe-Lys(Fmoc)-PACC-PNP 12.**

To a solution of **11** (800 mg, 0.851 mmol) in THF (15 mL) were added at 0 °C DIPEA (594 µL, 3.40 mmol), para-nitrophenyl chloroformate (515 mg, 2.55 mmol), and pyridine (17.3 µL, 0.213 mmol). The reaction mixture was stirred at room temperature for 2 h, after which dichloromethane (50 mL) and water (50 mL) were added. The aqueous layer was separated from the organic layer and extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with water, a saturated aqueous NaHCO₃ solution, and brine, dried with anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was washed with a 1:2 mixture of dichloromethane and diethyl ether. This gave **12** (812 mg, 0.734 mmol, 86%).

¹H NMR (300 MHz, DMSO-d₆) δ 0.95 (d, 3H, *J* = 7.8 Hz, CH₃ of Ala), 1.05 – 1.90 (m, 6H, 3 x CH₂ of Lys), 2.90 (dd, 1H, *J* = 11.4 Hz, *J* = 15.0 Hz, CH₂ of Phe), 3.11 (m, 2H, N-CH₂ of Lys), 3.26 (m, 1H, CH₂ of Phe), 4.24 – 4.89 (m, 9H, CH₂ and CH of Fmoc and 3 x H α), 5.24

(d, 2H, $J = 6.6$ Hz, CH=CH-CH₂), 6.81 (dt, 1H, $J = 17.4$ Hz, J= 7.0 Hz, CH=CH-CH₂), 7.23 (d, 1H, $J = 17.4$ Hz, CH=CH-CH₂), 7.66 – 8.25 (m, 23H, aromatic), 8.44 – 8.48 (m, 4H, aromatic), 8.97 (d, 2H, $J = 10.1$ Hz, aromatic) ppm; MS (FAB) m/e 1105 (M + H)⁺.

5

Example 7Synthesis of Fmoc-D-Ala-Phe-Lys(Fmoc)-PACC-DOX 13.

To a solution of **12** (100 mg, 90.5 μ mol) in N-methylpyrrolidinone (2 mL) were added triethylamine (13.2 μ L, 95.0 μ mol) and doxorubicin hydrochloride (55.1 mg, 95.0 μ mol). The reaction mixture was stirred at room temperature for 15 h and then poured into 10% isopropanol in ethyl acetate (25 mL). The resultant solution was washed with water, diluted with dichloromethane (50 mL), dried with anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO₂ – CH₂Cl₂/MeOH 93/7) and subsequent precipitation from diethyl ether, which gave **13** (82.3 mg, 54.5 μ mol, 60%).

¹H NMR (300 MHz, DMSO-d₆) δ 0.98 (d, 3H, $J = 6.8$ Hz, CH₃ of Ala), 1.12 (d, 3H, $J = 6.7$ Hz, 5'-Me), 1.24 – 2.25 (m, 8H, 3 x CH₂ of Lys and 2' and 8), 2.79 (m, 1H, CH₂ of Phe), 2.97 (m, 4H, N-CH₂ of Lys and 10), 3.11 (m, 1H, CH₂ of Phe), 3.47 (m, 1H, 4'), 3.74 (m, 1H, 3'), 20 3.97 (s, 3H, OCH₃), 3.97 – 4.37 & 4.54 – 4.60 & 4.72 & 4.85 & 4.97 & 5.24 & 5.47 (14H, CH₂ and CH of Fmoc and CH=CH-CH₂ and 1' and 5' and 7 and 14 and OH), 6.20 (m, 1H, CH=CH-CH₂), 6.55 (d, 1H, $J = 16.2$ Hz, CH=CH-CH₂), 7.14 – 7.93 (m, 32H, aromatic) ppm; MS (FAB) m/e 1533 (M + Na)⁺.

25

Example 8Synthesis of D-Ala-Phe-Lys-PACC-DOX (·2HCl) 14.

To a stirred solution of **50** (40.0 mg, 26.5 μ mol) in DMF (2 mL) was added piperidine (128 μ L, 1.30 mmol). After 10 min, the reaction mixture was slowly added to a stirred solution of ice-cold diethyl ether. The precipitate was collected by means of centrifugation, washed with diethyl ether, and dissolved in ethyl acetate. An approximately 0.5 M solution of HCl in ethyl acetate (200 μ L) was added and the precipitate formed was collected by means of

centrifugation. The residue was dissolved in a 2:1 mixture of *tert*-butanol and chloroform, and the resultant solution was concentrated under reduced pressure. This procedure was repeated twice, yielding **51** (29.4 mg, 25.8 μ mol, 97%) as an orange solid after freeze-drying.

¹H NMR (300 MHz, CDCl₃/CD₃OD) δ 1.22 (d, 3H, 5'-Me), 1.29 (d, 3H, CH₃ of Ala), 1.30 – 2.00 (m, 8H, 3 x CH₂ of Lys and 2'), 2.17 (br.d, 1H, 8), 2.39 (br.d, 1H, 8), 2.93 – 3.35 (m, 7H, CH₂ of Phe and N-CH₂ of Lys and 10 and 4'), 3.63 (m, 1H, 3'), 3.85 – 4.20 (m, 3H, 5' and 2 x H α), 4.07 (s, 3H, OCH₃), 4.50 – 4.78 (m, 5H, H α and 14 and CH=CH-CH₂), 5.25 (m, 1H, 1'), 5.48 (m, 1H, 7), 6.15 (m, 1H, CH=CH-CH₂), 6.56 (d, 1H, J = 14.6 Hz, CH=CH-CH₂), 7.23 – 7.56 (m, 10H, aromatic and 3), 7.82 (t, 1H, J = 8.0 Hz, 2), 8.01 (m, 1H, 1) ppm; MS (FAB) m/e 1065 (M + H)⁺.

Example 9

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABA 15.

15

A solution of 730 mg (1.37 mmol) protected tripeptide Aloc-D-Ala-Phe-Lys(Aloc)-OH **9** was dissolved in dry THF under an Argon atmosphere and cooled to –40 °C. NMM (166 μ l, 1.1 eq.) and isobutyl chloroformate (196 μ l, 1.1 eq.) were added. The reaction mixture was stirred for 3 hours at a temperature below –30 °C. A solution of 4-aminobenzyl alcohol (203 mg, 1.2 eq.) and NMM (181 μ l, 1.2 eq.) in dry THF was added dropwise to the reaction mixture. After 2 hours THF was evaporated and dichloromethane was added. The organic layer was washed with saturated sodium bicarbonate, a 0.5 N potassium bisulphate solution and brine, dried over anhydrous sodium sulphate, and evaporated. The residual pale yellow solid was purified by means of column chromatography (SiO₂ – CHCl₃/MeOH 9/1) to afford 812 mg (93%) of the desired product **15** as a cream coloured powder. M.P. 156 °C; ¹H-NMR (300 MHz, DMSO-D₆): δ 0.96 (d, 3H, CH₃-Ala), 1.10-1.85 (m, 6H, CH₂-Lys), 2.77 (dd, 1H, benzylic Phe), 2.97 (bd, 2H, N-CH₂-Lys), 3.09 (dd, 1H, benzylic Phe), 4.00 (t, 1H, H α), 4.20-4.60 (m, 8H, 2 H α and 4 Aloc and CH₂-OH), 5.00-5.35 (m, 4H, Aloc), 5.76-5.95 (m, 2H, Aloc), 7.05-7.30 (m, 7H, aromatic), 7.41 (d, 1H, NH), 7.56 (d, 2H, aromatic), 8.12 (d, 1H, NH), 8.18 (d, 1H, NH), 9.80 (s, 1H, NH anilide) ppm; MS (FAB) m/e 638 (M + H)⁺, 660 (M + Na)⁺; C₃₃H₄₃N₅O₈ (¹/₂ H₂O) calculated C 61.29%, H 6.86%, N 10.83%, measured C 61.39%, H 6.54%, N 10.55%.

Example 10Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PNP 16.

5 To a solution of 384 mg (0.602 mmol) **15** in dry THF/CH₂Cl₂ under an Argon atmosphere, 4-nitrophenyl chloroformate (182 mg, 1.5 eq.) and dry pyridine (73 µl, 1.5 eq.) were added. The reaction mixture was stirred at room temperature for 48 hours, then EtOAc was added. The organic layer was washed with 10% citric acid, brine and water, dried over anhydrous sodium sulphate and evaporated yielding a yellow solid. The product was purified by means of column chromatography (SiO₂ – CH₂Cl₂/MeOH 30/1) to afford 324 mg (67%) of carbonate **16**. ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.21 (d, 3H, CH₃-Ala), 1.25-2.05 (m, 6H, CH₂-Lys), 2.95 (dd, 1H, benzylic Phe), 3.13 (bt, 2H, N-CH₂-Lys), 3.27 (dd, 1H, benzylic Phe), 4.08 (dd, 1H, H α), 4.25 (dd, 1H, Hα), 4.30-4.65 (m, 5H, Hα and 4 Aloc), 5.04-5.35 (m, 4H, Aloc), 5.26 (s, 2H, CH₂-OH), 5.65-6.00 (m, 2H, Aloc), 7.10-7.35 (m, 5H, aromatic), 7.39-7.43 (2 * d, 4H, aromatic), 7.71 (d, 2H, aromatic), 8.28 (d, 2H, aromatic) ppm; MS (FAB) *m/e* 803 (M + H)⁺, 825 (M + Na)⁺.

Example 11Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABA 17.

20 To a solution of 156 mg (194 µmol) of compound **16** and 26.3 mg (1.1 eq.) PABA in dry N,N-dimethyl formamide under an Argon atmosphere was added diisopropylethyl amine (34 µl, 1.0 eq.) and a catalytic amount of N-hydroxybenzotriazole (7.9 mg, 0.3 eq.). The reaction solution 25 was stirred for 24 hours after which it was diluted with 10% propanol-2/EtOAc. The organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium hydrogensulfate and brine, dried over anhydrous sodium sulfate and evaporated to dryness. The yellow residual film was purified by means of column chromatography (SiO₂ - CHCl₃/MeOH 9/1) to yield 148 mg (97%) of the desired product **17**. M.P. 196-197°C; ¹H-NMR (300 MHz, CDCl₃): δ 1.20 (d, 3H, ³J=6.4 Hz, CH₃-Ala), 1.27-2.05 (m, 6H, 3 CH₂-Lys), 2.99 (dd, 1H, benzylic), 3.14 (m, 2H, N-CH₂-Lys), 3.27 (dd, 1H, benzylic), 4.00-4.64 (m, 7H, 3 Hα and Aloc), 4.57 (s, 2H, benzylic-spacer), 5.14 (s, 2H, benzylic-spacer), 5.06-5.37 (m, 4H, Aloc), 5.72 (m, 1H, Aloc), 5.88 (m, 1H, Aloc), 7.10-7.46 (m, 11H, aromatic), 7.64 (d, 2H, ³J=8.3 Hz, aromatic) ppm; MS

(FAB) m/e 809 ($M + Na$)⁺; $C_{41}H_{50}N_6O_{10}$ ($\cdot \frac{1}{2}H_2O$) calculated C 61.87%, H 6.46%, N 10.56%, measured C 61.84%, H 6.38%, N 10.38%.

Example 12

5

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PNP 18.

A solution of 80.2 mg (102 μ mol) of compound 17, pyridine (25 μ l, 3.0 eq.) and 4-nitrophenyl chloroformate (44.3 mg, 220 μ mol) in dry tetrahydrofuran/dichloromethane was stirred under 10 an Argon atmosphere at 0°C for two hours and overnight at room temperature. The solution was evaporated in vacuo and the residual product was dissolved in dichloromethane. After washing the organic layer with brine and 0.5 N potassium bisulfate, the organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The resulting crude product was subjected to column chromatography (SiO₂ - CHCl₃/MeOH 20/1) to obtain 61.9 mg (84%) of 15 compound 18. M.P. 69-70°C; ¹H-NMR (300 MHz, CDCl₃/CD₃OD) : δ 1.23 (d, 3H, ³J=7.0 Hz, CH₃-Ala), 1.10-2.08 (m, 6H, 3 CH₂-Lys), 3.04 (m, 1H, benzylic), 3.13 (m, 2H, N-CH₂-Lys), 3.27 (bd, 1H, benzylic), 4.06 (m, 1H, H α), 4.26 (m, 1H, H α), 4.35-4.70 (m, 5H, H α and Aloc), 5.04-5.47 (m, 4H, Aloc), 5.14 (s, 2H, benzylic-spacer), 5.24 (s, 2H, benzylic-spacer), 5.72 (m, 1H, Aloc), 5.90 (m, 1H, Aloc), 7.10-7.46 (m, 13H, aromatic), 7.65 (d, 2H, ³J=8.3 Hz, 20 aromatic), 8.27 (d, 2H, ³J=9.1 Hz, aromatic-PNP) ppm; MS (FAB) m/e 952 ($M + H$)⁺, 974 ($M + Na$)⁺; $C_{40}H_{46}N_6O_{12}$ ($\cdot \frac{1}{4}H_2O$) calculated C 59.51%, H 5.81%, N 10.41%, measured C 59.52%, H 5.54%, N 10.12%.

Example 13

25

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-DOX 19.

The double spacer containing 4-nitrophenyl carbonate 18 (140 mg, 0.147 mmol) and doxorubicin-HCl (94.1 mg, 1.1 eq.) in N-methyl pyrrolidinone were treated at room 30 temperature with triethyl amine (22.5 μ l, 1.1 eq.). The reaction mixture was stirred in the dark for 72 hours, again triethyl amine (1.1 eq.) was added and after an additional 24 hours the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water and brine, and was dried (Na₂SO₄). After evaporation of the solvents the crude

product was purified by means of column chromatography (chloroform-methanol; 9:1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate (chloroform-methanol; 9:1), to yield 72 mg (36%) of protected prodrug **19**. M.P. 129 °C; ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.22 (d, 3H, J = 7.1 Hz, sugar CH₃), 1.27 (d, 3H, J = 6.7 Hz, CH₃-Ala), 1.25-2.00 (m, 8H, CH₂-Lys and 2'), 2.15 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.04 (bd, 1H, J = 18.7 Hz, 10), 2.90-3.50 (m, 5H, benzylic Phe and N-CH₂-Lys and 10), 3.37 (bs, 1H, 4'), 3.58 (m, 1H, 3'), 3.85 (m, 1H, H_α), 4.08 (s, 3H, OMe), 4.14 (m, 1H, H_α), 4.29 (dd, 1H, 5'), 4.37-4.68 (m, 5H, H_α and 4 Aloc), 4.76 (s, 2H, 14), 4.96 (s, 2H, benzylic spacer), 5.11 (s, 2H, benzylic spacer), 5.02-5.40 (m, 4H, Aloc), 5.48 (bs, 1H, 1'), 5.61-6.00 (m, 3H, Aloc and 7), 7.08-7.39 (m, 9H, aromatic 5H Phe and 4H spacers), 7.33 (d, 2H, J = 8.3 Hz, 2H aromatic spacer), 7.42 (d, 1H, J = 8.4 Hz, 3), 7.62 (d, 2H, J = 8.0 Hz, 2H aromatic spacer), 7.80 (t, 1H, J = 8.1 Hz, 2), 8.03 (d, 1H, J = 7.5 Hz, 1) ppm; MS (FAB) m/e 1378 (M + Na)⁺; Anal. (C₆₉H₇₇N₇O₂₂·2H₂O) calculated C 59.52%, H 5.86%, N 7.04%, measured C 59.34%, H 5.71%, N 6.66%.

15

Example 14Synthesis of H-D-Ala-Phe-Lys-PABC-PABC-DOX-5.7HCl **20**.

20 To a solution of 48 mg (0.035 mmol) protected prodrug **19** in dry tetrahydrofuran/dichloromethane under an argon atmosphere was added morpholine (31 µl, 10 eq.) together with a catalytic amount of Pd(PPh₃)₄. The reaction mixture was stirred for one hour in the dark. The red precipitate was collected by means of centrifugation. Ethyl acetate was added and the mixture was acidified using 1.0 ml of 0.5 M hydrochloric acid/ethyl acetate. 25 The precipitate was collected by means of centrifugation and washed several times with ethyl acetate. Tert-butanol was added and evaporated and the resulting red film was freeze dried in water yielding 37 mg (83%) of prodrug **20**. Mp>300 °C; ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.20 (d, 3H, J = 7.0 Hz, sugar CH₃), 1.27 (d, 3H, J = 6.5 Hz, CH₃-Ala), 1.38-2.05 (m, 8H, CH₂-Lys and 2'), 2.18 (dd, 1H, 8), 2.36 (bd, 1H, 8), 2.82-3.41 (m, 6H, benzylic Phe and N-CH₂-Lys and 10), 3.37 (s, 1H, 4'), 3.60 (bs, 1H, 3'), 4.02 (m, 1H, H_α), 4.08 (s, 3H, OMe), 4.18 (m, 1H, H_α), 4.53 (dd, 1H, 5'), 4.66 (dd, 1H, H_α), 4.77 (s, 2H, 14), 4.95 (bs, 2H, benzylic spacer), 5.14 (s, 2H, benzylic spacer), 5.27 (bs, 1H, 1'), 5.48 (bs, 1H, 7), 7.09-7.50 (m, 11H, aromatic 5H Phe and 6H spacers and 3), 7.58 (d,

2H, $J = 8.4$ Hz, 2H aromatic spacer), 7.82 (t, 1H, $J = 8.0$ Hz, 2), 8.03 (d, 1H, $J = 7.6$ Hz, 1) ppm; MS (FAB) m/e 1188 ($M + H$)⁺, m/e 1210 ($M + Na$)⁺; Anal. (duplo) ($C_{61}H_{69}N_7O_{18}\cdot 5.7HCl$) calculated C 52.42%, H 5.39%, N 7.01%, measured C 52.38%, H 5.71%, N 7.14%.

5

Example 15Synthesis of 2'-[Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC]-paclitaxel 21.

- 10 4-Nitrophenyl carbonate **18** (47.4 mg, 49.8 μ mol) and paclitaxel (42.3 mg, 1.0 eq.) in dry tetrahydrofuran/dichloromethane under an Argon atmosphere were treated at room temperature with N,N-dimethyl-4-aminopyridine (DMAP) (6.7 mg, 1.1 eq.). The reaction mixture was stirred in the dark for 48 hours and was then concentrated to dryness. The product was dissolved in dichloromethane and the organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate and brine and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (SiO_2 - EtOAc/Hex/MeOH 5/5/1), to yield 67.5 mg (82%) of the desired protected paclitaxel prodrug **21**. M.P. 137-138°C; ¹H-NMR (300 MHz, $CDCl_3$): δ 1.14 (s, 3H, 17), 1.23 (s, 3H, 16), 1.27 (d, 3H, $^3J=7.1$ Hz, CH₃-Ala), 1.05-2.10 (m, 6H, CH₂-Lys), 1.67 (s, 3H, 19), 1.89 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.44 (s, 3H, 4-OAc), 2.97 (m, 1H, benzylic), 3.14 (m, 2H, N-CH₂-Lys), 3.21 (m, 1H, benzylic), 3.81 (d, 1H, $^3J=7.0$ Hz, 3), 4.03 (m, 1H, H α), 4.20 (d, 1H, $^2J=8.4$ Hz, 20b), 4.31 (d, 1H, $^2J=8.4$ Hz, 20a), 4.43 (m, 1H, 7), 4.34-4.74 (m, 6H, H α and Aloc), 4.90-5.37 (m, 11H, 2 H α , Aloc, 5 and 2 benzylic-spacer), 5.44 (d, 1H, $^3J=2.9$ Hz, 2'), 5.63 (m, 1H, Aloc), 5.69 (d, 1H, $^3J=7.1$ Hz, 2), 5.87 (m, 1H, Aloc), 5.97 (bd, 1H, $^3J=2.9$ Hz, $^3J=9.2$ Hz, 3'), 6.26 (m, 1H, 13), 6.29 (m, 1H, 10), 7.05-7.80 (m, 26H, aromatic), 8.14 (d, 2H, $^3J=7.2$ Hz, aromatic) ppm; MS (FAB) m/e 1668 ($M + H$)⁺, 1689 ($M + Na$)⁺; $C_{89}H_{99}N_7O_{25}\cdot 2H_2O$ calculated C 62.78%, H 6.10%, N 5.76%, measured C 62.55%, H 5.82%, N 5.57%.

Example 16Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-PABC]-paclitaxel (·2HCl) 22.

5 To a solution of 51.4 mg (30.8 μ mol) protected prodrug **21** in dry tetrahydrofuran under an Argon atmosphere was added glacial acetic acid (8.9 μ l, 5 eq.) together with tributyltinhydride (24.6 μ l, 3 eq) and a catalytic amount of Pd(PPh₃)₄. After 30 minutes the reaction mixture carefully 1 ml 0.5 M HCl/EtOAc was added to the reaction solution. The product was precipitated by addition of diethyl ether and the white precipitate was collected by means of centrifugation and washed several times with ether. Tert-butanol was added and evaporated again to remove an excess of HCl and the resulting product was dissolved in water and freeze dried yielding 46.9 mg (100%) of the desired prodrug **22**. M.P. > 192°C (dec.); ¹H-NMR (300 MHz, CDCl₃/CD₃OD) : δ 1.15 (s, 3H, 17), 1.21 (s, 3H, 16), 1.10-2.00 (m, 9H, CH₂-Lys and CH₃-Ala), 1.67 (s, 3H, 19), 1.90 (s, 3H, 18), 2.20 (s, 3H, 10-OAc), 2.43 (s, 3H, 4-OAc), 2.85 (m, 4H, benzylic and N-CH₂-Lys), 3.80 (d, 1H, ³J=6.9 Hz, 3), 4.24 (d, 1H, ²J=8.4 Hz, 20b), 4.31 (d, 1H, ²J=8.4 Hz, 20a), 4.39 (dd, 1H, 7), 4.56 (m, 1H, Ha), 5.68 (m, 1H, Ha), 4.98 (d, 1H, 5), 5.08 (m, 4H, 2 benzylic-spacer), 5.43 (d, 1H, ³J=2.7 Hz, 2'), 5.70 (d, 1H, ³J=7.0 Hz, 2), 5.97 (m, 1H, 3'), 6.22 (m, 1H, 13), 6.32 (m, 1H, 10), 7.05-7.68 (m, 24H, aromatic), 7.71 (d, 1H, ³J=7.2 Hz, aromatic), 8.14 (d, 2H, ³J=7.3 Hz, aromatic) ppm; MS (FAB) *m/e* 1499 (M + H)⁺, 1521 (M + Na)⁺; C₈₁H₉₁N₇O₂₁ (·3.7HCl) calculated C 59.60%, H 5.85%, N 6.01%, measured C 59.60%, H 5.88%, N 5.98%.

Example 1725 Synthesis of Fmoc-Trp-Lys(Boc)-OBu 24.

To a solution of 3.00 g (5.73 mmol) Fmoc-Trp-ONSu **23** in dry dichloromethane under an argon atmosphere were added at 0 °C 0.791 ml (1.00 equiv) triethylamine and 2.12 g (1.10 equiv) H-Lys (Boc)-OBu-HCl. The mixture was stirred at rt for 5 hours, then dichloromethane 30 was added and the organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water, dried over sodium sulfate and evaporated. The white solid **24** (3.52 g, 86%) was used without further purification. ¹H-NMR (300 MHz, CDCl₃): δ 1.10-1.92 (m, 24H, 3 CH₂-Lys and 18 *t*-Bu), 2.80-3.20 (m, 3H, N-CH₂-Lys and CH₂-Trp), 3.52 (d, 1H, CH₂-

Trp), 4.19 (t, 1H, Fmoc), 4.29-4.82 (m, 5H, 2 Fmoc, 2 H α and NH), 6.54 (d, H, Aryl), 7.06-7.76 (m, 12H, aromatic) ppm; MS (FAB) *m/e* 1444 (2M + Na); Anal. (C₄₁H₅₀N₄O₇·4H₂O) C, H, N calculated C 62.90%, H 6.30%, N 7.15%, measured C 63.22%, H 6.49%, N 7.13%.

5

Example 18Synthesis of Boc-D-Ala-Trp-Lys(Boc)-OBu 26.

3.52 g (4.95 mmol) of Fmoc-Trp-Lys(Boc)-OBu **24** was dissolved in 100 ml of
10 dioxane/methanol/2N sodium hydroxide (70/25/5) and stirred at rt for 1 hour. The mixture was neutralized with acetic acid (0.570 ml) and organic solvents were evaporated. Water and dioxane were added and the solution was freeze dried. Diisopropylether was added and after filtration the filtrate was evaporated. The product was dissolved in dry dichloromethane and added at 0 °C to a solution of 1.41 g (4.93 mmol) Boc-D-Ala-ONSu **6** and 0.756 ml (1.10
15 equiv) of triethylamine in dry dichloromethane. The mixture was stirred for 16 hours after which dichloromethane was added. The organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water, and dried over sodium sulfate and evaporated. The product was purified by means of column chromatography ((SiO₂ - first ethyl acetate/heptane
1/1 and then CHCl₃/MeOH; 9/1) to afford 2.26 g (3.42 mmol, 69%) of the tripeptide **26** as
20 white foam. ¹H-NMR (300 MHz, CDCl₃): δ 0.99-1.90 (m, 36 H, 3 CH₂-Lys, CH₃-Ala and 3 *t*-Bu), 2.80-3.50 (m, 4H, N-CH₂-Lys and 2 CH₂-Trp), 3.99 (m, 1H, H α), 4.33 (m, 1H, H α), 4.77
(br d, 1H, H α), 6.90-7.65 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 660 (M + H)⁺, 682 (M +
Na)⁺; Anal. (C₃₄H₅₃N₅O₈·H₂O) C, H, N calculated C 60.25%, H 8.17%, N 10.33%, measured
C 60.47%, H 8.08%, N 9.73%.

25

Example 19Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-OH 28.

30 2.56 g (4.13 mmol) Boc-D-Ala-Trp-Lys (Boc)-OBu (**26**) was stirred in a solution of hydrochloric acid in ethyl acetate (3M). After 5 hour the solvent was evaporated, tert-butanol was added and evaporated twice to remove remaining hydrochloric acid. The product was freeze dried in dioxane/water to yield a brown coloured powder.

To a solution of 706 mg (1.61 mmol) D-Ala-Phe-Lys-OH **27** in water/acetonitrile was added triethylamine until a pH of 9 – 9.5 was reached. Then a solution of 1.58 g (2.20 equiv) Aloc-ONSu in acetonitrile was added and the mixture was kept basic by adding triethylamine. After the pH of the mixture did not alter anymore, a 0.5 M solution of hydrochloric acid in ethyl acetate was added until a pH of 3 was reached. The mixture was thoroughly extracted with dichloromethane. The organic layer was washed with water, dried (Na_2SO_4) and evaporated. The cream coloured product **28** was used without further purification. $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.00-1.80 (m, 9H, 3 CH_2 -Lys and CH_3 -Ala), 2.80-3.35 (m, 4H, N- CH_2 -Lys and CH_2 Trp), 4.13 (m, 1H, $\text{H}\alpha$), 4.14 (m, 1H, $\text{H}\alpha$), 4.30-4.95 (m, 6H, 4 Aloc and 2 $\text{H}\alpha$), 5.01-5.40 (m, 5H, 4 Aloc and $\text{H}\alpha$), 5.70-6.30 (m, 3H, 2 Aloc and NH), 6.90-7.70 (m, 5H, aromatic) ppm; MS (FAB) m/e 572 ($\text{M} + \text{H}$) $^+$, 594 ($\text{M} + \text{Na}$) $^+$; Anal. ($\text{C}_{28}\text{H}_{37}\text{N}_5\text{O}_8 \cdot 1^{1/2}\text{H}_2\text{O}$) calculated C 56.18%, H 6.44%, N 11.70%, measured C 56.07%, H 6.22%, N 11.21%.

Example 20

15

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABA **29**.

A solution of 239 mg (0.419 mmol) Aloc-D-Ala-Trp-Lys(Aloc)-OH **28** was dissolved in dry tetrahydrofuran under an argon atmosphere and cooled to –40 °C. N-methylmorpholine (48.3 μ l, 1.05 equiv) and isobutylchloroformate (57.0 μ l, 1.05 equiv) were added. The reaction mixture was stirred for 2 hours at a temperature below –30 °C. A solution of 4-aminobenzyl alcohol (51.5 mg, 1.00 equiv) and N-methylmorpholine (50.6 μ l, 1.1 equiv) in dry THF was added dropwise to the reaction mixture. After 2 hours tetrahydrofuran was evaporated and dichloromethane was added. The organic layer was washed with saturated sodium bicarbonate, a 0.5 N potassium bisulphate solution and brine, dried (Na_2SO_4) and evaporated to afford 265 mg (94%) of the desired product **29** as a cream coloured powder. $^1\text{H-NMR}$ (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ 1.00-1.62 (m, 9H, CH_3 -Ala and 3 CH_2 -Lys), 2.90-3.70 (m, 4H, N- CH_2 -Lys and CH_2 -Trp), 4.48-4.92 (m, 7H, 2 $\text{H}\alpha$ and 4 Aloc), 4.72 (s, 2H, CH_2 -OH), 5.00-5.50 (m, 5H, 4 Aloc and $\text{H}\alpha$), 5.35-6.05 (m, 2H, Aloc), 6.80-7.83 (m, 9H, aromatic) ppm; MS (FAB) m/e 677 ($\text{M} + \text{H}$) $^+$, 699 ($\text{M} + \text{Na}$) $^+$.

Example 21

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PNP.

To a solution of 384 mg (0.602 mmol) of Aloc-D-Ala-Trp-Lys(Aloc)-PABA **29** in dry tetra hydro furan/dichloromethane under an argon atmosphere, 4-nitrophenylchloroformate (182 mg, 1.50 equiv) and dry pyridine (73 µl, 1.50 equiv) were added. The mixture was stirred at rt for 48 hours, and then ethyl acetate was added. The organic layer was washed with 10% citric acid, brine and water, dried (Na_2SO_4) and evaporated yielding a yellow solid. The product was purified by means of column chromatography (SiO_2 - $\text{CHCl}_3/\text{MeOH}$; 30/1) to afford 324 mg (67%) of carbonate Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PNP as a cream coloured powder. $^1\text{H-NMR}$ (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ 1.00-2.10 (m, 9H, CH_3 -Ala and 3 CH_2 -Lys), 2.90-3.70 (m, 4H, N- CH_2 -Lys and CH_2 -Trp), 3.64 (m, 1H, $\text{H}\alpha$), 3.81 (m, 1H, $\text{H}\alpha$), 4.38-4.81 (m, 5H, $\text{H}\alpha$ and 4 Aloc), 5.10-5.35 (m, 4H, Aloc), 5.21 (s, 2H, CH_2 -OH), 5.40-6.00 (m, 2H, Aloc), 7.00-7.85 (m, 11H, aromatic), 8.25 (d, 2H, $J = 8.1$, aromatic); MS (FAB) m/e 842 ($M + \text{H}$) $^+$, 864 ($M + \text{Na}$) $^+$.

Example 22Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABA **30**.

To a solution of 219 mg (260 µmol) of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PNP and 35.2 mg (1.1 equiv) 4-aminobenzyl alcohol in dry N,N-dimethylformamide under an Argon atmosphere was added diisopropylethylamine (45.3 µl, 1.00 equiv) and a catalytic amount of N-hydroxybenzotriazole (10.5 mg, 0.30 equiv). The reaction solution was stirred for 48 hours after which it was diluted with 10% propanol-2/EtOAc. The organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate and brine, dried over anhydrous sodium sulfate and evaporated to dryness. The pale yellow residual film was purified by means of column chromatography (SiO_2 - $\text{CHCl}_3/\text{MeOH}$ 15/1) to yield 192 mg (89%) of the desired product **30**. $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 0.90-2.10 (m, 9H, CH_3 -Ala and 3 CH_2 -Lys), 2.90-3.70 (m, 4H, N- CH_2 -Lys and CH_2 -Trp), 4.08 (m, 1H, $\text{H}\alpha$), 4.40-4.86 (m, 6H, 2 benzylic-spacer and 4 Aloc), 4.90-5.40 (m, 7H, 2 benzylic-spacer $\text{H}\alpha$ and Aloc), 5.50 (m, 1H, Aloc), 5.92 (m, 1H, Aloc), 6.72-7.82 (m, 13H, aromatic) ppm; MS (FAB) m/e 848 ($M + \text{Na}$) $^+$; ($\text{C}_{43}\text{H}_{51}\text{N}_7\text{O}_{10} \cdot 2^{3/4}\text{H}_2\text{O}$) calculated C 58.99%, H 6.50%, N 11.20%, measured C 59.15%, H 6.25%, N

11.15%.

Example 23

5 Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABC-PNP 31.

A solution of 70 mg (85 µmol) of compound **30**, pyridine (17 µl, 2.5 equiv) and 4-nitrophenylchloroformate (34 mg, 2.0 equiv) was stirred under an Argon atmosphere at 0°C for two hours and for 24 hours at room temperature. The solution was evaporated in vacuo and the residual product was dissolved in chloroform. After washing the organic layer with brine and 0.5 N potassium bisulfate, the organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The resulting crude product was subjected to column chromatography (SiO₂ - CHCl₃/MeOH 20/1) to obtain 54 mg (64%) of **31** as a pale yellow solid. ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 0.90-2.10 (m, 9H, CH₃-Ala and 3 CH₂-Lys), 2.90-3.10 (m, 4H, N-CH₂-Lys and CH₂-Trp), 3.27 (bd, 1H, benzylic), 4.35-4.78 (m, 6H, 2H_α and Aloc), 4.90-5.52 (m, 4H, Aloc), 5.13 (s, 2H, benzylic-spacer), 5.60 (m, 1H, Aloc), 5.94 (m, 1H, Aloc), 7.10-7.46 (m, 15H, aromatic), 8.36 (d, 2H, aromatic-PNP) ppm; MS (FAB) *m/e* 991 (M + H)⁺, 1013 (M + Na)⁺; C₅₀H₅₄N₈O₁₄ ·³/4H₂O calculated C 59.78%, H 5.57%, N 11.15%, measured C 60.12%, H 5.89%, N 10.76%.

20

Example 24

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABC-DOX 32.

25 The double spacer-containing 4-nitrophenylcarbonate **31** (41 mg, 0.041 mmol) and doxorubicin-HCl (26 mg, 1.1 equiv) in N-methylpyrrolidinone were treated at room temperature with triethylamine (6.3 µl, 1.1 equiv). The reaction mixture was stirred in the dark for 48 hours, again triethylamine (1.1 equiv) was added and after an additional 24 hours the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed 30 with water and brine, and was dried (Na₂SO₄). After evaporation of the solvents the crude product was purified by means of column chromatography (SiO₂ - CHCl₃/MeOH; 9/1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate (chloroform-methanol; 9/1), to yield 45 mg (78%) of the protected prodrug **32**. ¹H-NMR (300

MHz, CDCl₃/CD₃OD): δ 0.92-1.52 (m, 13H, sugar CH₃, CH₃-Ala, 3 CH₂-Lys and 2'), 2.15 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.18 (bd, 1H, 10), 2.90-3.10 (m, 5H, N-CH₂-Lys and CH₂-Trp and 10), 3.59 (bs, 1H, 4'), 3.82 (m, 1H, 3'), 3.85 (m, 1H, Hα), 4.11 (s, 3H, OMe), 4.21 (m, 1H, Hα), 4.45 (dd, 1H, 5'), 4.30-4.62 (m, 5H, Hα and 4 Aloc), 4.76 (s, 2H, 14), 4.96 (s, 2H, benzylic spacer), 5.11 (s, 2H, benzylic spacer), 5.513-5.4 (m, 2H, Aloc), 5.48 (bs, 1H, 1'), 5.58 (m, 2H, Aloc and 7), 5.91(m, 2H, Aloc), 6.70-7.39 (m, 11H, aromatic 5 Trp and 6 spacers), 7.41 (d, 1H, J = 8.4 Hz, 3), 7.63 (d, 2H, aromatic spacer), 7.78 (t, 1H, 2), 8.03 (d, 1H, J = 7.6 Hz, 1) ppm; MS (FAB) *m/e* 1417 (M + Na)⁺.

10

Example 25Synthesis of D-Ala-Trp-Lys-PABC-PABC-DOX (·7½ HCl) 33.

To a solution of 36 mg (0.026 mmol) protected prodrug 32 in dry THF/dichloromethane under 15 an argon atmosphere was added morpholine (22 µl, 10 equiv) together with a catalytic amount of Pd(PPh₃)₄. The reaction mixture was stirred for 1 hour in the dark. The red precipitate was collected by means of centrifugation. Ethyl acetate was added and the mixture was acidified using 0.5 ml of 1 M hydrochloric acid/ethyl acetate. The precipitate was collected by means of 20 centrifugation and washed several times with ethyl acetate. Tert-butanol was added and evaporated and the resulting red film was freeze dried in water yielding 28 mg (72%) of the doxorubicin prodrug 33. ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.10-1.96 (m, 13H, CH₃-Ala, CH₃-sugar, 3 CH₂-Lys and 2'), 2.09 (m, 1H, 8), 2.35 (bd, 1H, J = 15.1 Hz, 8), 2.79-3.39 (m, 3H, N-CH₂-Lys, CH₂-Trp and 10), 3.60 (s, 1H, 4'), 4.00 (bs, 1H, 3'), 4.09 (s, 3H, OMe), 4.54 (m, 1H, 5'), 4.77(s, 2H, 14), 4.97 (2 * d, 2H, Bn spacer), 5.13(s, CH₂, Bn spacer), 5.28 (bs, 1H, 1'), 5.48 (bs, 1H, 7), 6.99-7.72 (m, 12H, 5 Trp and 6 spacer), 7.62(d, 1H, 7.6 Hz, 3), 7.55 (d, 2H, J = 8.2 Hz, aromatic spacer), 7.83 (t, 1H, 2), 8.05 (d, 1H, J = 7.7 Hz, 1) ppm; MS (FAB) *m/e* 1228 (M + H)⁺; Anal. (C₆₃H₇₀N₈O₁₈·7 ^½HCl) calculated C 50.42%, H 5.21%, N 7.47%, measured C 50.56%, H 5.48%, N 7.35%.

30

Example 26

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABA 34.

100 mg (0.105 mmol) of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PNP **18** was dissolved in
5 dry N,N-dimethylformamide under an argon atmosphere and cooled to -8 °C. 4-
Aminobenzylalcohol (14.2 mg, 1.1 equiv), dipea (18.3 µl, 1.0 equiv) and 1-
hydroxybenzotriazole (HOBt) (4 mg, 0.3 equiv) were added. The reaction mixture was stirred
for 48 hours at room temperature, and diluted with 10% 2-propanol/ethyl acetate. The organic
layer was washed with water, saturated sodium bicarbonate, 0.5 N potassium bisulfate, and
10 brine, dried over sodium sulfate (Na_2SO_4), and evaporated to yield the desired product **34** as a
cream colored powder 86 mg (88%). ^1H NMR (300 MHz CDCl_3) δ 0.95-2.05 (m, 9H, 3CH_2 -
Lys and CH_3 -Ala), 2.88-3.11 (m , 4H, 2H Bn-Phe and N- CH_2 -Lys), 3.95-4.62 (m, 7H, $3\text{H}\alpha$
and 4H Aloc), 4.75 (s, 2H, CH_2 -OH), 5.12-5.21 (m, 6H, 4 Aloc and CH_2 -Bn), 5.09 (s, 2H,
15 CH_2 -Bn), 5.65-6.00 (m, 2H, Aloc), 6.79-7.41 (m, 15H, aromatic) 7.62 (d, 2H, aromatic) ppm;
MS (FAB) m/e 959 ($M + \text{Na}^+$).

Example 27Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABC-PNP 35.

20 To a solution of 59 mg (0.063 mmol) of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABA **34**
in dry tetrahydrofuran and dichloromethane under an argon atmosphere, were added at -40 °C
respectively pyridine (13 µl, 2.5 equiv) and 4-nitrophenyl chloroformate (25 mg, 2.0 equiv).
After stirring for 4.5 hours at -40 °C and overnight at 6 °C, pyridine (10 µl, 2.0 equiv) and 4-
25 nitrophenylchloroformate (25 mg, 2.0 equiv) were added again. This was repeated after 48
hours stirring at 6 °C. After another 48 hours the solution was evaporated in vacuo and the
residual product was dissolved in chloroform. The organic layer was washed with 10% citric
acid, brine and water, dried over sodium sulfate (Na_2SO_4) and evaporated yielding a yellow
solid. The crude product was purified by means of column chromatography (SiO_2 - CHCl_3
30 / MeOH ; 15/1) to give the desired product **35** quantitatively. $^1\text{H-NMR}$ (300 MHz,
 $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ 1.12-1.89 (m, 9H, CH_3 -Ala and 3 CH_2 -Lys), 3.04 (m, 1H, benzylic), 3.14
(m, 2H, N- CH_2 -Lys), 3.27 (bd, 1H, benzylic), 4.09 (m, 1H, $\text{H}\alpha$), 4.28 (m, 1H, $\text{H}\alpha$), 4.34-4.68
(m, 5H, $\text{H}\alpha$ and Aloc), 5.02-5.40 (m, 4H, Aloc), 5.14 (s, 2H, benzylic-spacer), 5.21 (s, 2H,

benzylic-spacer), 5.31 (s, 2H, benzylic-spacer), 5.72 (m, 1H, Aloc), 5.90 (m, 1H, Aloc), 7.10-7.52 (m, 17H, aromatic), 7.63 (d, 2H, J=8.3 Hz, aromatic), 8.27 (d, 2H, J=9.1 Hz, aromatic-PNP) ppm; MS (FAB) *m/e* 1102 (M + H)⁺, 1124 (M + Na)⁺.

5

Example 28Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABC-DOX 36.

The 4-nitrophenyl carbonate **35** (69 mg, 0.063 mmol) and doxorubicin-HCl (40 mg, 1.1 equiv) in N-methylpyrrolidinone were treated at room temperature with triethylamine (9.7 µl, 1.1 equiv). The reaction mixture was stirred in the dark for 24 hours and the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water and brine, and was dried over sodium sulfate (Na₂SO₄). After evaporation of the solvents the crude product was purified by means of column chromatography (SiO₂- CHCl₃/MeOH 9/1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate (CHCl₃/MeOH; 9/1), to yield 65 mg (71%) of the protected prodrug **36**. ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.10-1.80 (m, 14H, sugar CH₃, CH₃-Ala, 3 CH₂-Lys and 2'), 2.14 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.18 (bd, 1H, 10), 2.82-3.41 (m, 6H, benzylic Phe and N-CH₂-Lys and 10), 3.37 (s, 1H, 4'), 3.60 (bs, 1H, 3'), 4.02 (m, 1H, H_α), α), 4.07 (s, 3H, OMe) 4.29 (dd, 1H, 5'), 4.37-4.68 (m, 5H, H_α and 4 Aloc), 4.76 (s, 2H, 14), 4.95 (bs, 2H, benzylic spacer), 5.10 (s, 2H, benzylic spacer), 5.14 (s, 2H, benzylic spacer), 5.02-5.35 (m, 4H, Aloc), 5.27 (bs, 1H, 1'), 5.47 (bs, 1H, 7), 5.70 (m, 1H, Aloc), 5.89 (m, 1H, Aloc), 7.09-7.50 (m, 16H, 5 Phe and 10 spacers and 3), 7.64 (d, 2H, J = 8.4 Hz, 2H aromatic spacer), 7.79 (t, 1H, J = 8.1 Hz, 2), 8.06 (d, 1H, J = 7.5 Hz, 1) ppm; MS (FAB) *m/e* 1506 (M + H)⁺, 1528 (M + Na)⁺.

25

Example 29Synthesis of D-Ala-Phe-Lys-PABC-PABC-PABC-DOX (·2HCl) 37.

To a solution of 40 mg protected prodrug **36** (0.027 mmol) in dry tetrahydrofuran/dichloromethane under an argon atmosphere were added morpholine (24 µl, 10 equiv) and a catalytic amount of Pd(PPh₃)₄. The reaction mixture was stirred for 1 hour in the dark. The red precipitate was collected by means of centrifugation and washed several

times with ethyl acetate. Water and dioxane were added and the mixture was acidified using 4.4 ml of 0.125 mM hydrochloric acid. After freeze drying 26 mg (70%) of doxorubicin prodrug 37 was obtained. ¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.19 (d, 3H, J = 6.9 Hz, sugar CH₃), 1.27 (d, 3H, J = 6.6 Hz, CH₃-Ala), 1.25-2.00 (m, 8H, 3 CH₂-Lys and 2'), 2.18 (dd, 1H, 8), 2.33 (br d, 1H, J = 16.1 Hz, 8), 2.89-3.38 (m, 6H, N-CH₂-Lys and 10 and Bn Phe), 3.60 (s, 1H, 4'), 3.72 (m, 1H, 3'), 4.08 (s, 3H, OMe), 4.18 (m, 1H, H α), 4.53 (dd, 1H, 5') 4.66 (m, 1H, H α), 4.77 (s, 2H, 14), 4.96 (s, 2H, Bn spacer), 5.11 (s, 2H, bn spacer), 5.17 (s, 2H, Bn spacer), 5.27 (br s, 1H, 1'), 5.48 (br s, 1H, 7), 7.05-7.35 (m, 16H, aromatic spacer and 3), 7.52 (d, 2H, J = 8.5 Hz, aromatic spacer), 7.84 (t, 1H, 2), 8.01 (d, 1H, J = 7.7 Hz, 1) ppm.

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Example 30Synthesis of 2'-[4-nitrophenyl carbonate]-paclitaxel 38.

15 To a solution of 194 mg (0.227 mmol) paclitaxel in dry dichloromethane under an Argon atmosphere was added pyridine (4 drops). At -50 °C, 275 mg (6.0 eq.) 4-nitrophenyl chloroformate dissolved in dry dichloromethane was added. The reaction mixture was stirred at -50 °C and after 4 hours 4-nitrophenyl chloroformate (4.2 eq.) was added. After 1 hour the mixture was diluted with dichloromethane and washed with 0.5 N potassium bisulfate and 20 brine and dried over anhydrous sodium sulphate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (SiO₂ - EtOAc/Hex 1/1), to yield 133 mg of activated paclitaxel 38 (78%, 73% conversion). M.P. 161°C; ¹H-NMR (300 MHz, CDCl₃): δ 1.14 (s, 3H, 17), 1.25 (s, 3H, 16), 1.68 (s, 3H, 19), 1.92 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.49 (s, 3H, 4-OAc), 2.55 (m, 1H, 6a), 3.82 (d, 1H, 3), 4.21 (d, 1H, 20b), 4.32 (d, 1H, 20a), 4.42 (m, 1H, 7), 4.96 (bd, 1H, 5), 5.53 (d, 1H, 2'), 5.69 (d, 1H, 2), 6.09 (q, 1H, 3'), 6.29 (s, 1H, 10), 6.34 (m, 1H, 13), 6.90 (d, 1H, N-H), 7.20-7.65 (m, 13H, aromatic), 7.75 (d, 2H, aromatic), 8.15 (d, 2H, aromatic), 8.25 (d, 2H, nitrophenyl) ppm; MS (FAB) m/e 1020 (M + H)⁺, 1042 (M + Na)⁺; C₅₄H₅₄N₂O₁₉ (·1½H₂O) calculated C 62.00%, H 5.40%, N 2.68%, measured C 61.89%, H 5.52%, N 2.64%.

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Example 31Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-N(Me)-(CH₂)₂-N(Me)CO]-paclitaxel (·2HCl) 43.5 Step a: Synthesis of N(Me)-(CH₂)₂-N(Me)-Z 39 (Z = benzyloxycarbonyl).

To a solution of 1.21 g (13.7 mmol) N,N'-dimethyl ethylenediamine in dry dichloromethane under an Argon atmosphere at room temperature was added dropwise a solution of Z-ONSu (338 mg, 1.36 mmol) in dry dichloromethane. After stirring for 120 minutes the solution was 10 concentrated in vacuo. The residual product was dissolved in ethyl acetate and the organic layer was washed with brine. The organic solvent was dried over anhydrous sodium sulfate and evaporated to dryness. The oily product was purified by means of column chromatography (SiO₂ - CHCl₃/MeOH 1/1) to obtain 249 mg (83%) of the product 39 as an oil. ¹H-NMR (300 MHz, CDCl₃): δ 2.42 (bd, 3H, ³J=13.9 Hz, CH₃-NH-CH₂), 2.73 (m, 2H, CH₃-NH-CH₂), 2.95 15 (s, 3H, CH₃-N), 3.41 (bs, 2H, CH₂-N), 5.13 (s, 2H, CH₂-Z), 7.25-7.40 (m, 5H, aromatic) ppm.

Step b: Synthesis of 2'-[Z-N(Me)-(CH₂)₂-N(Me)CO]-paclitaxel 40.

To a solution of 114 mg (112 µmol) 2'-activated paclitaxel 38 and 25 mg Z-protected N,N'- 20 dimethyl ethylenediamine 39 in dry dichloromethane under an Argon atmosphere at -50°C was added triethyl amine (20.0 µl, 144 µmol). The solution was stirred 7 hours at -40°C, subsequently allowed to heat up to room temperature and then stirring was continued overnight at room temperature. The solution was diluted with dichloromethane and washed with saturated sodium bicarbonate, brine and 0.5 N potassium bisulfate. The organic layer was 25 dried over anhydrous sodium sulfate and concentrated in vacuo to obtain a yellow film. The product was purified by column chromatography (SiO₂ - EtOAc/Hex 2/1) to obtain 113 mg (92%) of the desired product 40. M.P. 130-131°C; ¹H-NMR (300 MHz, CDCl₃): δ 1.12 (s, 3H, 17), 1.21 (s, 3H, 16), 1.70 (s, 3H, 19), 2.00 (s, 3H, 18), 2.26 (s, 3H, 10-OAc), 2.60 (s, 3H, 4-OAc), 2.90 (s, 3H, CH₃-spacer), 2.94 (s, 3H, CH₃-spacer), 2.97 (m, 1H, CH₂-spacer), 3.06 30 (m, 1H, CH₂-spacer), 3.54 (m, 1H, CH₂-spacer), 3.78 (m, 1H, CH₂-spacer), 3.84 (d, 1H, ³J=7.2 Hz, 3), 4.23 (d, 1H, ²J=8.4 Hz, 20b), 4.32 (d, 1H, ²J=8.4 Hz, 20a), 4.47 (m, 1H, 7), 4.69 (d, 1H, ²J=12.4 Hz, benzylic), 4.85 (d, 1H, ²J=12.4 Hz, benzylic), 5.01 (m, 1H, 5), 5.47 (d, 1H, ³J=2.9 Hz, 2'), 5.68 (d, 1H, ³J=7.0 Hz, 2), 6.19 (dd, 1H, ³J=9.8 Hz, ³J=2.9 Hz, 3'), 6.28 (s, 1H,

10), 6.33 (m, 1H, 13), 6.94-7.70 (m, 16H, aromatic), 7.83 (d, 2H, $^3J=7.3$ Hz, aromatic), 8.16 (d, 2H, $^3J=7.1$ Hz, aromatic), 8.57 (d, 1H, $^3J=9.8$ Hz, NH) ppm; MS (FAB) m/e 1102 (M + H) $^+$, 1124 (M + Na) $^+$; $C_{60}H_{67}N_3O_{17}\cdot H_2O$ calculated C 64.33%, H 6.21%, N 3.73%, measured C 64.65%, H 6.11%, N 3.76%.

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Step c: Synthesis of 2'-[N(Me)-(CH₂)₂-N(Me)CO]-paclitaxel ($\cdot 7$ AcOH) 41.

To a solution of 61.8 mg (56.1 μ mol) of **40** in 5% acetic acid/methanol was added a catalytic amount of 10% Pd-C. The mixture was stirred for 1 hour under a H₂ atmosphere. The Pd-C 10 was removed by means of centrifugation, methanol was evaporated in vacuo, and ethyl acetate was added. The organic layer was extracted with water. The water layer was freeze dried yielding 78.0 mg (100%) of the desired product **41**.

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Step d: Synthesis of 2'-[Aloc-D-Ala-Phe-Lys(Aloc)-PABC-N(Me)-
(CH₂)₂-N(Me)CO]-paclitaxel 42.

To a solution of 152 mg (95.8 μ mol) of paclitaxel-spacer compound **41** and 80.7 mg (101 μ mol) of carbonate **16** in dry tetrahydrofuran under an Argon atmosphere was added triethyl amine (200 μ l, 1.44 mmol). After 24 hours the solution was concentrated to dryness and the 20 residual product was dissolved in dichloromethane and washed with saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product was subjected to column chromatography (SiO₂ - EtOAc/Hex/MeOH 5/5/1) to obtain 113 mg (72%) of the desired protected prodrug **42**. M.P. 127-128⁰C; ¹H-NMR (300 MHz, CDCl₃): δ 1.13 (s, 3H, 17), 1.22 (s, 3H, 16), 1.27 (d, 3H, $^3J=5.6$ Hz, CH₃-Ala), 1.04-2.00 (m, 6H, CH₂-Lys), 1.69 (s, 3H, 19), 2.00 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.59 (s, 3H, 4-OAc), 2.90 (s, 3H, CH₃-spacer), 2.91 (s, 3H, CH₃-spacer), 2.76-25 3.46 (m, 6H, CH₂-spacer, benzylic and N-CH₂-Lys), 3.54 (m, 1H, CH₂-spacer), 3.74 (m, 1H, CH₂-spacer), 3.84 (d, 1H, $^3J=7.0$ Hz, 3), 4.00-5.00 (m, 3H, 3 H α), 4.23 (d, 1H, $^2J=8.4$ Hz, 20b), 4.32 (d, 1H, $^2J=8.4$ Hz, 20a), 4.48 (m, 1H, 7), 4.62 (d, 1H, $^2J=12.3$ Hz, benzylic), 4.83 (d, 1H, $^2J=12.4$ Hz, benzylic), 4.30-4.73 (m, 4H, Aloc), 4.93-5.39 (m, 5H, Aloc and 5), 5.48 (d, 1H, $^3J=2.9$ Hz, 2'), 5.69 (d, 1H, $^3J=7.0$ Hz, 2), 5.54-5.78 (m, 1H, Aloc), 5.88 (m, 1H, Aloc), 6.18 (bd, 1H, 3'), 6.30 (s, 1H, 10), 6.33 (m, 1H, 13), 7.05-7.78 (m, 20H, aromatic), 7.82 (d, 2H, $^3J=7.4$ Hz, aromatic), 8.16 (d, 2H, $^3J=7.2$ Hz, aromatic) ppm; MS (FAB) m/e 1653 (M

+ Na⁺; C₈₆H₁₀₂N₈O₂₄ calculated C 62.61%, H 6.35%, N 6.79%, measured C 62.40%, H 6.31%, N 6.36%.

Step e: Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-N(Me)-(CH₂)₂-N(Me)CO]-

5 **paclitaxel (·2HCl) 43.**

To a solution of 83.0 mg (50.9 μmol) protected prodrug 42 in dry tetrahydrofuran under an Argon atmosphere was added glacial acetic acid (12 μl, 4.0 eq.) together with tributyltinhydride (41 μl, 3.0 eq) and a catalytic amount of Pd(PPh₃)₄. After 30 minutes the product was precipitated by addition of diethyl ether. The white precipitate was collected by means of centrifugation and washed several times with diethyl ether. Tert-butanol was added and evaporated again to remove an excess of HCl and the resulting product was dissolved in water/dioxane and freeze dried yielding 56 mg (70%) of prodrug 43. M.P. 142⁰C; ¹H-NMR (300 MHz, CDCl₃): δ 1.13 (s, 3H, 17), 1.21 (s, 3H, 16), 1.26 (d, 3H, ³J=6.6 Hz, CH₃-Ala), 10 1.05-2.00 (m, 6H, CH₂-Lys), 1.69 (s, 3H, 19), 2.00 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.58 (s, 3H, 4-OAc), 2.89 (s, 3H, CH₃-spacer), 2.91 (s, 3H, CH₃-spacer), 2.67-3.64 (m, 3H, CH₂-spacer), 2.95 (m, 1H, benzylic), 3.07 (m, 2H, N-CH₂-Lys), 3.15 (m, 1H, benzylic), 3.78 (m, 1H, CH₂-spacer), 3.83 (d, 1H, ³J=7.1 Hz, 3), 4.10-5.05 (m, 2H, 2 H_α), 4.22 (d, 1H, ²J=8.4 Hz, 20b), 4.32 (d, 1H, ²J=8.4 Hz, 20a), 4.46 (m, 1H, 7), 4.60 (m, 1H, H_α), 4.65 (d, 1H, ²J=12.3 Hz, 20) 15 benzylic-spacer), 4.80 (d, 1H, ²J=12.4 Hz, benzylic-spacer), 4.99 (bd, 5H, ³J=7.4 Hz, 5), 5.47 (d, 1H, ³J=2.9 Hz, 2'), 5.68 (d, 1H, ³J=6.9 Hz, 2), 6.17 (bd, 1H, ³J=2.9 Hz, ³J=9.6 Hz, 3'), 6.30 (s, 1H, 10), 6.31 (m, 1H, 13), 7.05-7.70 (m, 20H, aromatic), 7.82 (d, 2H, ³J=7.5 Hz, aromatic), 8.16 (d, 2H, ³J=7.2 Hz, aromatic), 8.54 (d, 1H, ³J=9.6 Hz, NH-paclitaxel) ppm; MS (FAB) m/e 20 1463 (M + H)⁺, 1485 (M + Na)⁺; C₈₅H₉₇N₇O₂₂ (·3AcOH) calculated C 61.04%, H 6.50%, N 6.71%, measured C 60.91%, H 6.45%, N 7.10%.

Example 32

Synthesis of 2'-O-[D-Ala-Phe-Lys-PABC-PABC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel·2HCl.

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Step a: **Synthesis of 2'-O-[Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel.**

To a solution of paclitaxel-spacer conjugate **41** (50 mg, 48.6 μ mol) and peptide-spacer conjugate **18** (46.3 mg, 48.6 μ mol) in THF (3 mL) was added triethylamine (101 μ L, 0.730 mmol). The reaction mixture was stirred at room temperature for 15 h and then concentrated under reduced pressure. The residue was dissolved in dichloromethane, and the solution was washed with a saturated aqueous NaHCO₃ solution and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Column chromatography (SiO₂ - EtOAc/Hex/MeOH 5/4/1) gave **44** (58.2 mg, 32.7 μ mol, 67%).

¹H-NMR (300 MHz, CDCl₃): δ 1.12 (s, 3H, 17), 1.21 (s, 3H, 16), 1.26 (d, 3H, J = 6.6 Hz, CH₃ of Ala), 1.05 – 2.00 (m, 6H, 3 x CH₂ of Lys), 1.69 (s, 3H, 19), 1.99 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.58 (s, 3H, 4-OAc), 2.90 (s, 3H, N-CH₃), 2.91 (s, 3H, N-CH₃), 2.80-3.85 (m, 9H, N-CH₂-CH₂-N and CH₂ of Phe and N-CH₂ of Lys and 3), 4.00 – 5.38 (m, 19H, 3 x H α and 20 and 7 and 2 x CH₂ of spacer and 5 and 2 x CH₂=CH-CH₂), 5.46 (d, 1H, J = 2.7 Hz, 2'), 5.60 (m, 1H, CH₂=CH-CH₂), 5.69 (d, 1H, J = 6.9 Hz, 2), 5.89 (m, 1H, CH₂=CH-CH₂), 6.16 (dd, 1H, J = 9.3 Hz, J = 2.4 Hz, 3'), 6.30 (s, 1H, 10), 6.31 (m, 1H, 13), 7.09 – 7.81 (m, 26H, aromatic), 8.16 (d, 2H, J = 7.2 Hz, aromatic) ppm.

Step b: Synthesis of 2'-O-[D-Ala-Phe-Lys-PABC-PABC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel·2HCl.

To a solution of protected prodrug **44** (50.0 mg, 28.1 μ mol) in dry THF (3 mL) were added tributyltin hydride (22.7 μ L), Pd(PPh₃)₄ (6.5 mg, 5.6 μ mol), and acetic acid (6.5 μ L, 0.112 mmol). After 30 min, the reaction mixture was slowly added to cold diethyl ether. The white precipitate was collected by means of centrifugation and washed two times with diethyl ether. The residue was suspended in ethyl acetate and a 0.5 M HCl solution in ethyl acetate (0.5 mL) was added under vigorous stirring. The white precipitate was collected by means of centrifugation and washed two times with ethyl acetate. *tert*-Butyl alcohol was added to the residue and subsequently evaporated to remove excess HCl. The residue was dissolved in water en freeze-dried, giving **45** (32.0 mg, 19.0 μ mol, 68%).

¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.17 (s, 3H, 17), 1.19 (s, 3H, 16), 1.26 (d, 3H, J = 6.9 Hz, CH₃ of Ala), 1.20 – 2.00 (m, 6H, 3 x CH₂ of Lys), 1.70 (s, 3H, 19), 2.02 (s, 3H, 18), 2.21 (s, 3H, 10-OAc), 2.55 (s, 3H, 4-OAc), 2.80 – 2.99 (m, 4H, CH₂ of Phe and N-CH₂ of Lys), 2.93 (s, 6H, 2 x N-CH₃), 3.10 – 3.87 (m, 4H, N-CH₂-CH₂-N), 3.86 (d, 1H, J = 6.9 Hz, 3), 4.06 (q, 1H, J = 7.0 Hz, H α), 4.26 – 4.76 & 5.02 (m, 8H, 2 x H α and 20 and 7 and CH₂ of spacer

and 5), 5.16 (s, 2H, CH₂ of spacer), 5.45 (d, 1H, *J* = 2.5 Hz, 2'), 5.72 (d, 1H, *J* = 7.1 Hz, 2), 6.10 (m, 1H, 3'), 6.26 (m, 1H, 13), 6.40 (s, 1H, 10), 7.08 – 7.62 (m, 24H, aromatic), 7.77 (d, 2H, *J* = 7.6 Hz, aromatic), 8.14 (m, 2H, aromatic) ppm.

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Example 33Synthesis of 2'-O-[D-Ala-Phe-Lys-PACC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel·2HCl 49.Step a: Preparation of NZ-D-Ala-Phe-Lys(NZ)-OH 9c.

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To a solution of tripeptide **8** (506 mg, 1.39 mmol) in dichloromethane (10 mL) were added trimethylsilyl chloride (0.568 mL, 4.44 mmol) and DIPEA (0.509 mL, 2.92 mmol). The reaction mixture was stirred at reflux temperature for 1.5 h. Then, the reaction mixture was cooled down to 0 °C, after which DIPEA (776 µL, 4.44 mmol) and para-nitrobenzyl chloroformate (NZ-Cl) (629 mg, 2.92 mmol) were added. The reaction mixture was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and acetate buffer (pH = 5). The organic layer was washed with more acetate buffer, water, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Column chromatography (SiO₂ – CH₂Cl₂/MeOH/AcOH 90/7/5) gave **9c** (620 mg, 0.858 mmol, 61%).

¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.18 (d, 3H, *J* = 7.1 Hz, CH₃ of Ala), 1.36 – 1.97 (m, 6H, 3 x CH₂ of Lys), 2.94 (dd, 1H, CH₂ of Phe), 3.12 – 3.23 (m, 3H, CH₂ of Phe and N-CH₂ of Lys), 4.12 (m, 1H, H α), 4.42 (m, 1H, H α), 4.63 (m, 1H, H α), 5.17 (m, 4H, CH₂ of NZ), 7.17 – 7.25 (m, 5H, aromatic), 7.51 (d, 4H, *J* = 8.2 Hz, aromatic), 8.19 (d, 4H, *J* = 8.0 Hz, aromatic) ppm.

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Step b: Preparation of NZ-D-Ala-Phe-Lys(NZ)-PACA 46.

To a solution of protected tripeptide **9c** (300 mg, 0.415 mmol) in THF (10 mL) were added at –40 °C *N*-methylmorpholine (50.2 µL, 0.457 mmol) and isobutyl chloroformate (62.4 mg, 0.457 mmol). The reaction mixture was stirred at –30 °C for 3 h. Then, spacer **10** (77.3 mg, 0.498 mmol) and *N*-methylmorpholine (55.0 µL, 0.498 mmol) in THF (5 mL) were added. The reaction mixture was stirred for 15 h, the reaction temperature slowly being raised to room

temperature. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane. The solution was washed with a saturated aqueous NaHCO₃ solution, a 0.5 M aqueous KHSO₄ solution, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Column chromatography (SiO₂ – CHCl₃/MeOH 93/7) gave **46** (267 mg, 0.313 mmol, 75%).

¹H-NMR (300 MHz, CDCl₃/CD₃OD): δ 1.22 (d, 3H, J = 7.2 Hz, CH₃ of Ala), 1.40 – 2.04 (m, 6H, 3 x CH₂ of Lys), 2.96 (dd, 1H, J = 9.9 Hz, J = 14.4 Hz, CH₂ of Phe), 3.15 (m, 2H, N-CH₂ of Lys), 3.30 (dd, 1H, J = 14.4 Hz, J = 4.2 Hz, CH₂ of Phe), 4.08 (m, 1H, H α), 4.23 (dd, 2H, J = 1.1 Hz, J = 5.6 Hz, CH₂ of spacer), 4.46 (m, 1H, H α), 4.56 (m, 1H, H α), 4.90 (d, 1H, J = 13.8 Hz, CH₂ of NZ), 4.99 (d, 1H, J = 13.8 Hz, CH₂ of NZ), 5.17 (s, 2H, CH₂ of NZ), 6.26 (dt, 1H, J = 5.7 Hz, J = 15.9 Hz, CH=CH-CH₂), 6.52 (d, 1H, J = 15.9 Hz, CH=CH-CH₂), 7.22 – 7.32 (m, 9H, aromatic), 7.53 (d, 2H, J = 8.6 Hz, aromatic), 7.61 (d, 2H, J = 8.6 Hz, aromatic), 8.04 (d, 2H, J = 8.6 Hz, aromatic), 8.19 (d, 2H, J = 8.6 Hz, aromatic) ppm.

15 Step c: Preparation of NZ-D-Ala-Phe-Lys(NZ)-PACC-PNP 47.

To a solution of peptide-spacer **46** (244 mg, 0.286 mmol) in THF (15 mL) were added DIPEA (0.216 mL, 1.24 mmol), para-nitrophenyl chloroformate (187 mg, 0.927 mmol), and pyridine (6.3 μ L, 77.2 μ mol). The reaction mixture was stirred for 48 h and then diluted with ethyl acetate (50 mL). The solution was washed with a 10% aqueous citric acid solution, water, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Column chromatography (SiO₂ – CHCl₃/MeOH 95/5) gave **47** (291 mg, 0.286 mmol, 100%).

¹H-NMR (300 MHz, CDCl₃/CD₃OD/DMSO-d₆): δ 1.23 (d, 3H, J = 7.1 Hz, CH₃ of Ala), 1.38 – 2.03 (m, 6H, 3 x CH₂ of Lys), 2.97 (dd, 1H, J = 9.9 Hz, J = 14.0 Hz, CH₂ of Phe), 3.14 (m, 2H, N-CH₂ of Lys), 3.28 (dd, 1H, CH₂ of Phe), 4.10 (m, 1H, H α), 4.47 (m, 1H, H α), 4.54 (m, 1H, H α), 4.87 – 5.00 (m, 4H, CH₂ of spacer and CH₂ of NZ), 5.17 (s, 2H, CH₂ of NZ), 6.27 (dt, 1H, J = 6.8 Hz, J = 15.4 Hz, CH=CH-CH₂), 6.70 (d, 1H, J = 15.4 Hz, CH=CH-CH₂), 7.22 – 7.33 (m, 9H, aromatic), 7.47 (d, 2H, J = 9.2 Hz, aromatic), 7.53 (d, 2H, J = 8.6 Hz, aromatic), 7.67 (d, 2H, J = 8.6 Hz, aromatic), 8.04 (d, 2H, J = 8.6 Hz, aromatic), 8.18 (d, 2H, J = 8.6 Hz, aromatic), 8.30 (d, 2H, J = 9.2 Hz, aromatic) ppm.

Step d: Preparation of 2'-O-[NZ-D-Ala-Phe-Lys(NZ)-PACC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel 48.

To a solution of paclitaxel-spacer conjugate **41** (50 mg, 48.6 µmol) and peptide-spacer conjugate **47** (54.5 mg, 53.5 µmol) in THF (3 mL) was added triethylamine (101 µL, 0.730 mmol). The reaction mixture was stirred at room temperature for 15 h and then concentrated under reduced pressure. The residue was dissolved in dichloromethane, and the solution was washed with a saturated aqueous NaHCO₃ solution and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Column chromatography (SiO₂ - EtOAc/Hex/MeOH 5/4/1) gave **48** (65.7 mg, 35.6 µmol, 73%).

¹H-NMR (300 MHz, CDCl₃): δ 1.12 (s, 3H, 17), 1.21 (s, 3H, 16), 1.30 (d, 3H, J = 7.0 Hz, CH₃ of Ala), 1.20 – 2.10 (m, 6H, 3 x CH₂ of Lys), 1.68 (s, 3H, 19), 2.00 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.58 (s, 3H, 4-OAc), 2.91 (s, 3H, N-CH₃), 2.95 (s, 3H, N-CH₃), 2.90 – 3.90 (m, 8H, N-CH₂-CH₂-N and CH₂ of Phe and N-CH₂ of Lys), 3.83 (d, 1H, J = 6.6 Hz, 3), 4.10 – 5.00 (m, 11H, 3 x H_α and 20 and 5 and 7 and CH=CH-CH₂ and CH₂ of NZ), 5.16 (s, 2H, CH₂ of NZ), 5.46 (d, 1H, J = 3.3 Hz, 2'), 5.68 (d, 1H, J = 7.5 Hz, 2), 6.01 – 6.17 (m, 2H, 3' and CH=CH-CH₂), 6.31 (s, 1H, 10), 6.32 (m, 1H, 13), 6.40 (d, 1H, J = 15.9 Hz, CH=CH-CH₂), 7.19 – 7.70 (m, 24H, aromatic), 7.83 (d, 2H, J = 7.2 Hz, aromatic), 8.01 (d, 2H, J = 8.7 Hz, aromatic), 8.15 – 8.19 (m, 4H, aromatic) ppm.

20

Step e: Preparation of 2'-O-[D-Ala-Phe-Lys-PACC-N(Me)-(CH₂)₂-N(Me)CO]paclitaxel·2HOAc 49.

To a solution of protected prodrug **48** (50.0 mg, 27.1 µmol) in methanol (5 mL) was added acetic acid (1.5 mL) and zinc (88.5 mg, 1.35 mmol). The resultant suspension was stirred at room temperature for 24 h. Then, the reaction mixture was filtered over HYFLO. Water was added and methanol was evaporated under reduced pressure. The resultant solution was freeze-dried to obtain a slightly yellow solid, which was dissolved in a mixture of dichloromethane and methanol (2 mL, 1:1 v/v). This solution was added to cold diisopropyl ether. The white precipitate was collected by means of centrifugation and washed two times with diisopropyl ether, which afforded prodrug **49** (27.8 mg, 17.3 µmol, 64%).

Example 34Stability of both double spacer containing paclitaxel prodrugs 22 and 43.

5 The prodrugs were incubated at concentrations of 150 µM in 0.1 M Tris/HCl buffer (pH 7.3) for 3 days and showed no formation of degradation products (TLC, RP₁₈; CH₃CN/H₂O/AcOH 19/19/2).

Stability of the double spacer containing doxorubicin prodrug 20.

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The prodrug was incubated at a concentration of 100 – 270 µM in 0.1 M Tris/HCl buffer (pH 7.3) for 90 hours and showed no formation of degradation products (TLC, RP₁₈; CH₃CN/H₂O/AcOH 19/19/2).

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Example 35Enzymatic hydrolysis of the double spacer containing prodrugs by plasmin.

Hydrolysis of the doxorubicin prodrugs was investigated by incubation at a prodrug 20 concentration of 100 µM in 0.1 M Tris/hydrochloric acid buffer (pH 7.3) in the presence of 50 or 20 µg/mL human plasmin (Fluka). Analysis was carried out with the following HPLC system using a Chrompack Microsphere-C18 column (3 µm, 2 × 100 × 4.6 mm). Elution of the analytical column was performed using 7:3 methanol/50 mM Et₃N-formate buffer (pH 3.0). Detection was performed using an UV-detector ($\lambda = 500$ nm).

25

	[prodrug] (µM)	[plasmin] (µg/mL)	T _½ activation (min)
Prodrug 44	100	50	19
Prodrug 44	200	20	> 75
Prodrug 20	200	20	12

Hydrolysis of the paclitaxel prodrugs was investigated by incubation at a prodrug concentration of 200 µM in 0.1 M Tris/hydrochloric acid buffer (pH 7.3) in the presence of 100 µg/mL human plasmin (Fluka). All double spacer containing paclitaxel prodrugs were

converted to yield the corresponding parent drug. Capillary electrophoresis was carried out with a CE Ext. Light Path Capillary (80.5 cm, 50 µm), with 1:1 methanol/0.05 M sodium phosphate buffer (pH 7.0) as eluent. Detection was performed at 200 and 254 nm.

	[prodrug] (µM)	[plasmin] (µg/mL)	T _½ activation (min)	T _½ cyclisation (min)
Prodrug 45	200	100	42	
Prodrug 43	200	100	4	
Prodrug 22	200	100	7.5	47

5

Example 36

Cytotoxicity.

- 10 The anti-proliferative effect of prodrugs and parent drugs was determined *in vitro* applying seven well-characterised human tumor cell lines and the microculture sulphorhodamine B (SRB) test. The anti-proliferative effects were determined and expressed as IC₅₀ values, that are the (pro)drug concentrations that gave 50% inhibition when compared to control cell growth after 5 days of incubation.

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Table 1. ID₅₀ values^{a,b} (ng/ml) of prodrugs and parent drugs.

Cell Line:	MCF-7	EVSA-T	WIDR	IGROV	M19	A498	H226
Prodrug 20	242	546	627	896	302	2303	503
Prodrug 43	60	119	117	499	96	681	62
Prodrug 22	11	5	5	22	7	25	7
Paclitaxel	<3	<3	<3	10	<3	<3	<3
Doxorubicin	10	8	11	60	16	90	199

^a Drug dose that inhibited cell growth by 50% compared to untreated control cultures. ^b SRB cell viability test.

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Cell lines: MCF-7; breast cancer. EVSA-T; breast cancer. WIDR; colon cancer. IGROV; ovarian cancer. M19; melanoma. A498; renal cancer. H226; non-small cell lung cancer.

CLAIMS**1. A compound of the formula:**

5

 $V-(W)_k-(X)_l-A-Z$ **wherein:**

V is a specifier which is removed by an enzyme, optionally after prior binding to a receptor;

- 10 **(W)_k-(X)_l-A** is an elongated self-eliminating spacer system;
W and **X** are each a 1,(4+2n) electronic cascade spacer, being the same or different;

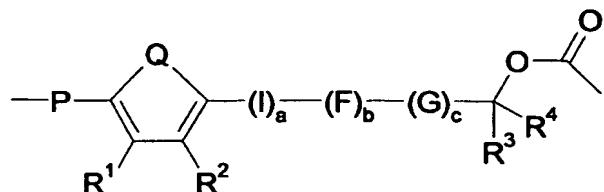
- A** is either a spacer group of formula $(Y)_m$, wherein
Y is a 1,(4+2n) electronic cascade spacer, or a group of formula
15 **U** being a cyclisation elimination spacer;
Z is a therapeutic or diagnostic moiety;
k, **l** and **m** are independently an integer from 0 (included) to
5 (included);
n is an integer of 0 (included) to 10 (included),

- 20 **with the provisos that:**

- when **A** is $(Y)_m$: $k+l+m \geq 1$, and
 - if $k+l+m=1$, then $n > 1$;
- when **A** is **U**: $k+l \geq 1$.

- 25 **2. The compound of claim 1, wherein group U is an ω -amino aminocarbonyl cyclisation spacer, and Z is a moiety coupled to U via its hydroxyl group.**

- 3. The compound of claims 1 and 2, wherein the electronic cascade spacers W, X and Y are independently selected from compounds having the formula:**



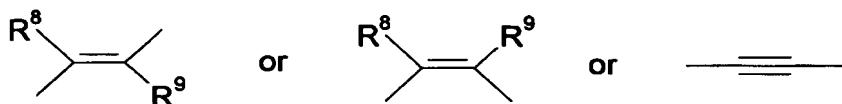
$Q = -R^5C=CR^6-$, S, O, NR^5 , $-R^5C=N-$, or $-N=CR^5-$
 $P = NR^7$, O, S

wherein

a, b, and c are independently an integer of 0 to 5;

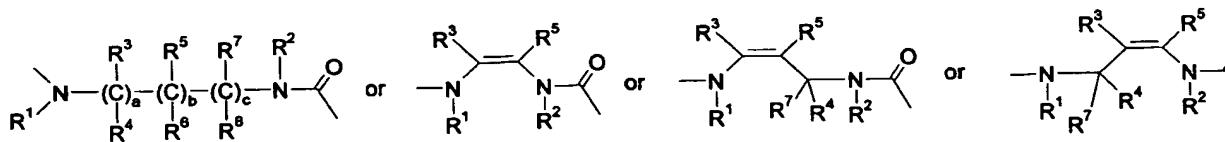
I, F and G are independently selected from compounds having the formula:

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wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , and R^9 independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphoxy (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonooxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where
10 R_x, R_x¹ and R_x² are independently selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, two or more of the substituents R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, or R⁹ optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.
15

20 4. The compound of claims 1 and 2, wherein the ω -amino aminocarbonyl cyclisation elimination spacer U is a compound having the formula:



wherein:

5 a is an integer of 0 or 1; and

b is an integer of 0 or 1; and

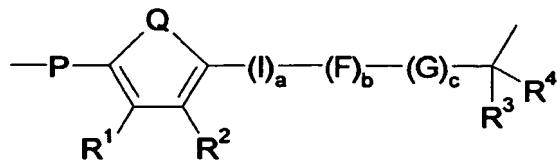
c is an integer of 0 or 1; provided that

a + b + c = 2 or 3;

- 10 and wherein R¹ and/or R² independently represent H, C₁₋₆ alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), 15 carboxylate (COOR_x), sulphonyl (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group; and
- R³, R⁴, R⁵, R⁶, R⁷, and R⁸ independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OR_x), sulphoxy (S(=O)OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group; and
- 20 wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, and R⁸ can be a part of one or more aliphatic or aromatic cyclic structures, two or more of the substituents R¹, R², R³, R⁴, R⁵, R⁶, R⁷, or R⁸ optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.
- 25

30

5. The compound of claim 1 wherein A is an electronic cascade spacer having the structural formula:



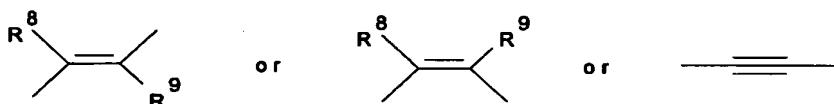
$Q = -R^5C=CR^6-$, S, O, NR^5 , $-R^5C=N-$, or $-N=CR^5-$
 $P = NR^7$, O, S

wherein

a, b, and c are independently an integer of 0 to 5;

I, F and G are independently selected from compounds having the formula:

5



wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , and R^9 independently represent H, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphoxy (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphixy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where

15 R_x , R_x^1 and R_x^2 are independently selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, two or more of the substituents R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

20 6. The compound of claim 5 wherein Z is a moiety that is coupled to A via its phenolic hydroxyl group.

7. The compound of any of claims 1 to 5 wherein the specifier V contains a substrate that can be cleaved by plasmin.

8. The compound of any of claims 1 to 5 wherein the specifier V contains a substrate that can be cleaved by a cathepsin.
9. The compound of any of claims 1 to 5 wherein the specifier V contains a substrate that can be cleaved by cathepsin B.
10. The compound of any of claims 1 to 5 wherein the specifier V contains a substrate that can be cleaved by β -glucuronidase, prostate-specific antigen (PSA), urokinase-type plasminogen activator (u-PA), or a member of the family of matrix metalloproteinases.
11. The compound of any of claims 1 to 5 wherein the specifier V contains a nitro-(hetero)aromatic moiety that can be removed by reduction under hypoxic conditions or by reduction by a nitroreductase.
12. The compound of any of claims 1 to 5 wherein the spacer system $(W)_k-(X)_l-A$ is p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl, p-aminobenzylloxycarbonyl-p-amino-benzylloxycarbonyl-p-aminobenzylloxycarbonyl, p-aminocinnamylloxycarbonyl, p-amino-cinnamylloxycarbonyl-p-aminobenzylloxycarbonyl, p-aminobenzylloxycarbonyl-p-amino-cinnamylloxycarbonyl, p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl, p-amino-phenylpentadienyloxycarbonyl, p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl-oxycarbonyl, p-aminophenylpentadienyloxycarbonyl-p-aminobenzylloxycarbonyl, p-amino-phenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl, p-aminobenzyl-oxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminocinnamylloxycarbonyl(methyl-amino)ethyl(methylamino)carbonyl, p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-(methylamino)ethyl(methylamino)carbonyl, p-aminocinnamylloxycarbonyl-p-aminobenzyl-oxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminobenzylloxycarbonyl-p-amino-cinnamylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl, p-aminobenzyl-oxycarbonyl-p-aminobenzyl, p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzyl, p-aminocinnamyl, p-aminocinnamylloxycarbonyl-p-aminobenzyl, p-aminobenzylloxycarbonyl-p-aminocinnamyl, p-aminocinnamylloxycarbonyl-p-aminocinnamyl, p-aminophenylpentadienyl, p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl, p-

aminophenylpentadienyloxycarbonyl-p-aminobenzyl, or p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

13. The compound of any of claims 1 to 5 wherein the moiety Z is an anticancer agent.

5

14. The compound of claim 13 wherein the said moiety Z is

the amino containing cytotoxic moiety daunorubicin, doxorubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, mitomycin C, mitomycin A, 9-amino camptothecin, aminopterin, actinomycin, bleomycin, N⁸-acetyl spermidine, 1-(2-chloroethyl)-

10 1,2-dimethanesulfonyl hydrazine, tallysomycin, or derivatives thereof,

the hydroxyl containing cytotoxic moiety etoposide, camptothecin, irinotecan, topotecan, 9-amino camptothecin, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, doxorubicin, morpholine-doxorubicin, N-(5,5-diacetoxypentyl) doxorubicin, vincristine, vinblastine, or derivatives thereof,

15 the sulphydryl containing cytotoxic moiety esperamicin, 6-mercaptopurine, or derivatives thereof,

the carboxyl containing cytotoxic moiety methotrexate, camptothecin (ring-opened form of the lactone), butyric acid, retinoic acid, or derivatives thereof.

20 15. The compound of any of claims 1 to 4 wherein the moiety Z represents the anticancer drug paclitaxel or a paclitaxel derivative that is coupled to the promoiety V-(W)_k-(X)_l-U- via its 2'-hydroxyl group.

16. The compound of any of claims 1 to 9 wherein the specifier V is a tripeptide.

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17. The compound of claim 16 wherein the covalent linkage of said tripeptide specifier moiety to its immediately adjacent moiety is at the C-terminus of said tripeptide specifier moiety.

18. The compound of claim 17 wherein the C-terminal amino acid residue is arginine or lysine,

30 the middle amino acid residue is selected from the group consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, cyclohexylglycine, tryptophan and proline, and the N-terminal amino acid residue is a D-amino acid residue, a protected L-amino acid residue, or protected glycine.

19. The compound of any of claims 1 to 7 wherein the specifier V is D-alanylphenylalanyllysine, D-valylleucyllysine, D-alanylleucyllysine, D-valylphenylalanyllysine, D-valyltryptophanyllysine, or D-alanyltryptophanyllysine.

5

20. The compound of any of claims 1 to 6, 8, 9, wherein the specifier V is an amino-terminal capped peptide covalently linked at its C-terminus to its immediately adjacent moiety.

21. The compound of claim 20 wherein the said amino-terminal capped peptide specifier is
- 10 benzyloxycarbonylphenylalanyllysine, benzyloxycarbonylvalyllysine, D-phenylalanylphenylalanyllysine, benzyloxycarbonylvalylcitrulline, tert-butylloxycarbonylphenylalanyllysine, benzyloxycarbonylalanylarginylarginine, benzyloxycarbonylphenylalanyl-N-tosylarginine, 2-aminoethylthiosuccinimidopropionylvalylcitrulline, 2-
- 15 aminoethylthiosuccinimidopropionyllsylphenylalanyllysine, acetylphenylalanyllysine, or benzyloxycarbonylphenylalanyl-O-benzoylthreonine.

22. The compound of any of claims 1 to 4, 7 to 9 which is N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)doxorubicin, N-(D-alanylphenylalanyl-20 lysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-doxorubicin, N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)daunorubicin, N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)daunorubicin, N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)mitomycin C, N-(D-alanylphenylalanyl-25 lysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-mitomycin C, N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-9-aminocamptothezin, N-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-9-aminocamptothezin, 2'-O-(D-alanyl-phenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 2'-O-(D-30 alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 2'-O-(D-alanyl-

phenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, 5 O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-10 aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)-9-amino- camptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzyl- oxycarbonyl-p-aminobenzylloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-amino- benzylloxycarbonyl-p-aminobenzylloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-15 aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)- topotecan, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)topotecan, N-(D-alanylphenylalanyllysyl-p-amino- cinnamyloxycarbonyl)doxorubicin, N-(D-alanylphenylalanyllysyl-p-aminocinnamyloxy- 20 carbonyl-p-aminobenzylloxycarbonyl)doxorubicin, N-(D-alanylphenylalanyllysyl-p-amino- cinnamyloxycarbonyl)daunorubicin, N-(D-alanylphenylalanyllysyl-p-aminocinnamyloxy- carbonyl-p-aminobenzylloxycarbonyl)daunorubicin, N-(D-alanylphenylalanyllysyl-p-amino- cinnamyloxycarbonyl)mitomycin C, N-(D-alanylphenylalanyllysyl-p-aminocinnamyl- oxycarbonyl-p-aminobenzylloxycarbonyl)mitomycin C, N-(D-alanylphenylalanyllysyl-p- 25 aminocinnamyloxycarbonyl)-9-aminocamptothecin, N-(D-alanylphenylalanyllysyl-p- aminocinnamyloxycarbonyl-p-aminobenzylloxycarbonyl)-9-aminocamptothecin, 2'-O-(D- alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl)paclitaxel, 2'-O-(D-alanylphenyl- alanyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzylloxycarbonyl)paclitaxel, 7-O-(D- alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl)paclitaxel, 7-O-(D-alanylphenylalanyl- 30 lysyl-p-aminocinnamyloxycarbonyl-p-aminobenzylloxycarbonyl)paclitaxel, 2'-O-(D-alanyl- phenylalanyllysyl-p-aminocinnamyloxycarbonyl)docetaxel, 2'-O-(D-alanylphenylalanyllysyl- p-aminocinnamyloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, 7-O-(D-alanylphenyl- alanyllysyl-p-aminocinnamyloxycarbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-amino-

cinnamylloxycarbonyl-p-aminobenzylloxycarbonyl)docetaxel, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminobenzylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-amino-5 cinnamylloxycarbonyl-p-aminobenzylloxycarbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-amino-cinnamylloxycarbonyl-p-aminobenzylloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminocinnamyl-10 oxycarbonyl-p-aminobenzylloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-amino-cinnamylloxycarbonyl)topotecan, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminobenzylloxycarbonyl)topotecan, N-(D-alanylphenylalanyllysyl-p-aminocinnamyl-15 carbonyl-p-aminocinnamylloxycarbonyl)doxorubicin, N-(D-alanylphenylalanyllysyl-p-amino-cinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)daunorubicin, N-(D-alanylphenylalanyl-lysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)mitomycin C, N-(D-15 alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)-9-aminocamptothecin, 2'-O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-amino-cinnamylloxycarbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminocinnamyl-20 carbonyl-p-aminocinnamylloxycarbonyl)paclitaxel, 2'-O-(D-alanylphenylalanyllysyl-p-amino-cinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)docetaxel, 7-O-(D-alanylphenylalanyl-25 lysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)docetaxel, O-(D-alanyl-phenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)-9-30 aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-amino-cinnamylloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminocinnamyl-oxycarbonyl-p-aminocinnamylloxycarbonyl)topotecan, N-(D-alanylphenylalanyllysyl-p-amino-phenylpentadienyloxy carbonyl)doxorubicin, N-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy-35 carbonyl)daunorubicin, N-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy-oxycarbonyl)mitomycin C, N-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyl-40 oxycarbonyl)-9-aminocamptothecin, 2'-O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy carbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy-45 carbonyl)paclitaxel, 2'-O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy carbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxy carbonyl)docetaxel,

O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxycarbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxycarbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxycarbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxycarbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminophenylpentadienyloxycarbonyl)topotecan, 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)-carbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)topotecan, 2'-O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 2'-O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminocinnamylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)camptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)topotecan, 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 7-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)carbonyl)paclitaxel,

amino)ethyl(methylamino)carbonyl)docetaxel, 7-O-(D-alanylphenylalanyllysyl-p-amino-benzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)-docetaxel, O-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl(methylamino)ethyl(methylamino)carbonyl)camptothecin, O-(D-alanylphenyl-5 alanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl-(methylamino)carbonyl)-9-aminocamptothecin, O-(D-alanylphenylalanyllysyl-p-aminobenzyl-oxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)etoposide, O-(D-alanylphenylalanyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-(methylamino)ethyl(methylamino)carbonyl)irinotecan, O-(D-alanylphenylalanyllysyl-p-10 aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)-carbonyl)topotecan, N-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl)doxorubicin, N-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl-p-aminobenzyloxycarbonyl)doxorubicin, N-(D-valylleucylllysyl-p-aminobenzyloxy-carbonyl-p-aminobenzyloxycarbonyl)daunorubicin, N-(D-valylleucylllysyl-p-aminobenzyloxy-15 carbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)daunorubicin, N-(D-valyl-leucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)mitomycin C, N-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl)mitomycin C, N-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl)-9-aminocamptothecin, N-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-20 aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-9-aminocamptothecin, 2'-O-(D-valyl-leucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 2'-O-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl)paclitaxel, 7-O-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl)paclitaxel, 7-O-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-25 carbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 2'-O-(D-valylleucylllysyl-p-aminobenzyloxy-carbonyl-p-aminobenzyloxycarbonyl)docetaxel, 2'-O-(D-valylleucylllysyl-p-aminobenzyloxy-carbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)docetaxel, 7-O-(D-valyl-leucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxy-carbonyl-p-aminobenzyloxycarbonyl)docetaxel, 7-O-(D-valyl-leucylllysyl-p-aminobenzyloxy-carbonyl-p-30 carbonyl)docetaxel, O-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyl-oxycarbonyl)camptothecin, O-(D-valylleucylllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyl-oxycarbonyl-p-aminobenzyloxycarbonyl)camptothecin, O-(D-valylleucylllysyl-p-aminobenzyl-oxycarbonyl-p-aminobenzyloxycarbonyl)-9-aminocamptothecin, O-(D-valylleucylllysyl-p-

aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-9-amino-camptothecin, O-(D-valylleucyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)-etoposide, O-(D-valylleucyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)etoposide, O-(D-valylleucyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)irinotecan, O-(D-valylleucyllysyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl)irinotecan, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl)doxorubicin, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)doxorubicin, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)daunorubicin, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)daunorubicin, N-(D-valylleucyllysyl-p-aminocinnamyl-oxycarbonyl)mitomycin C, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)mitomycin C, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)-9-aminocamptothecin, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyl-oxycarbonyl)-9-aminocamptothecin, 2'-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)-paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 7-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)paclitaxel, 7-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)docetaxel, 2'-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)docetaxel, 7-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)docetaxel, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)-camptothecin, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxy-carbonyl)camptothecin, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)-9-amino-camptothecin, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxy-carbonyl)-9-aminocamptothecin, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)-etoposide, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)-etoposide, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl)irinotecan, O-(D-valyl-leucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)irinotecan, O-(D-valyl-leucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)topotecan, O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl)topotecan, N-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl)doxorubicin, N-(D-valylleucyllysyl-p-

aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)daunorubicin, N-(D-valyl-leucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)mitomycin C, N-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)-9-amino-camptothecin, 2'-O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)paclitaxel, 7-O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)docetaxel, 7-O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)docetaxel, O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)camptothecin, O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)-9-aminocamptothecin, O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)etoposide, O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)irinotecan, O-(D-valylleucyllysyl-p-aminocinnamylloxycarbonyl-p-aminocinnamylloxycarbonyl)topotecan, N-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)doxorubicin, N-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)mitomycin C, N-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)-9-aminocamptothecin, 2'-O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)-paclitaxel, 7-O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)docetaxel, 7-O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)docetaxel, O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)camptothecin, O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)-9-aminocamptothecin, O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)etoposide, O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)irinotecan, O-(D-valylleucyllysyl-p-aminophenylpentadienyloxy carbonyl)topotecan, 2'-O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 7-O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, 7-O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)camptothecin, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)-9-aminocamptothecin, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)etoposide, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)

(methylamino)carbonyl)irinotecan, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-
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5 paclitaxel, 2'-O-(D-valylleucyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl-
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(methylamino)ethyl(methylamino)carbonyl)docetaxel, O-(D-valylleucyllysyl-p-amino-
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(methylamino)ethyl(methylamino)carbonyl)irinotecan, O-(D-valylleucyllysyl-p-amino-
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ethyl(methylamino)carbonyl)docetaxel, 7-O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-p-
aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)docetaxel, O-(D-
20 valylleucyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl-
(methylamino)carbonyl)camptothecin, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-p-
aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)-9-aminocamptothecin,
O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)-
ethyl(methylamino)carbonyl)etoposide, O-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-p-
25 aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)irinotecan, O-(D-valyl-
leucyllysyl-p-aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl(methylamino)ethyl-
(methylamino)carbonyl)topotecan, or a salt thereof

23. The compound of claim 22 which is N-(D-alanylphenylalanyllysyl-p-
30 aminobenzylloxycarbonyl-p-aminobenzylloxycarbonyl)doxorubicin

24. The compound of claim 22 which is N-(D-valylleucyllysyl-p-aminobenzylloxycarbonyl-p-
aminobenzylloxycarbonyl)doxorubicin

25. The compound of claim 22 which is N-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl)doxorubicin

5 26. The compound of claim 22 which is N-(D-valylleucyllsyl-p-aminocinnamyloxycarbonyl)doxorubicin

27. The compound of claim 22 which is 2'-O-(D-alanylphenylalanyllysyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel

10

28. The compound of claim 22 which is 2'-O-(D-valylleucyllsyl-p-aminobenzylloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel

29. The compound of claim 22 which is 2'-O-(D-alanylphenylalanyllysyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel
15

30. The compound of claim 22 which is 2'-O-(D-valylleucyllsyl-p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl)paclitaxel

20 31. The compound of any of the preceding claims wherein the moiety Z is an antibiotic, an anti-inflammatory agent, or an anti-viral agent.

32. The compound of any of the preceding claims wherein the specifier V contains a polymer.

25 33. The composition comprising more than one compound of any of the preceding claims, connected with a polymeric structure.

34. The compound of any of the preceding claims, wherein the specifier V is removed by an enzyme that is transported to the vicinity of target cells or target tissue via ADEPT, PDEPT,
30 VDEPT, or GDEPT.

35. The process for the synthesis of prodrugs of any of the preceding claims having at least one electronic cascade spacer group, and an ω -amino aminocarbonyl cyclisation elimination

spacer group, connected to each other, incorporated between a specifier group and a drug molecule such that said drug molecule is connected to said cyclisation elimination spacer group, via the hydroxyl functionality of the drug molecule, by coupling a first electronic cascade spacer group, connected to said specifier group, if desired via at least one, second 5 electronic cascade spacer group, being the same or different as said first electronic cascade spacer group, to said cyclisation elimination spacer group.

36. The process of claim 35, wherein said drug molecule is paclitaxel, and in a first step a cyclisation elimination spacer group is coupled to paclitaxel via its 2'-hydroxyl group through 10 a carbamate linkage via addition of the free spacer-amine to a 4-nitrophenyl carbonate activated drug, followed by deprotection to obtain a first fragment consisting of a cyclisation spacer connected with paclitaxel, and in a second step one or more 1,(4+2n) electronic cascade spacers, being the same or different, wherein n is an integer of 0 to 10, are coupled to a specifier group, subsequently activated to the corresponding 4-nitrophenyl carbonate, 15 whereafter in a third step the fragments obtained in the first and second step are coupled to one another under basic reaction conditions.

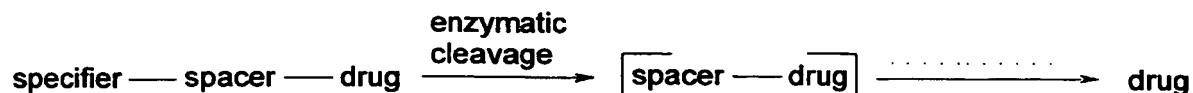
37. The process for the synthesis of prodrugs of any of claims 1 to 5 in which electronic cascade spacers are connected to one another by coupling of the terminal alcohol group of an 20 electronic cascade spacer to the aniline amino group of another electronic cascade spacer through a carbamate linkage by conversion of the alcohol group of the first-mentioned electronic cascade spacer to the corresponding 4-nitrophenyl carbonate and reacting this molecule with the other electronic cascade spacer in the presence of a catalytic amount of 1-hydroxybenzotriazole either in the presence or absence of base.

38. Use of compounds of any of claims 1 to 34 for the preparation of a pharmaceutical preparation for the treatment of a mammal being in need thereof.

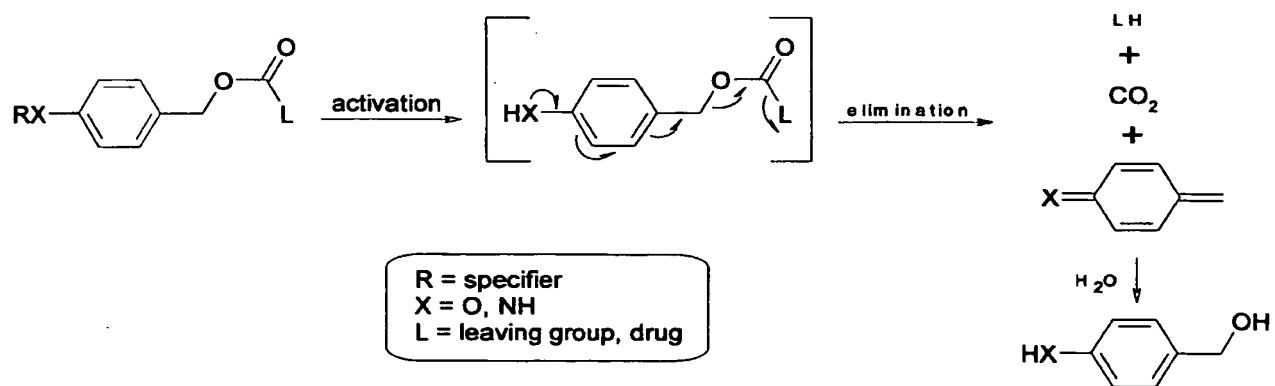
39. A method of treating a mammal being in need thereof, whereby the method comprises the administration of a pharmaceutical composition to the mammal in a therapeutically effective dose.

40. A process for preparing a pharmaceutical composition containing a compound of any of claims 1 to 34, to provide a solid or a liquid formulation for administration orally, topically or by injection.

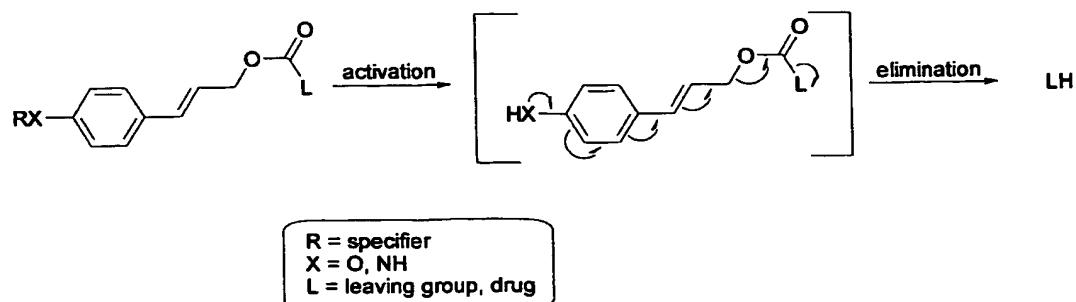
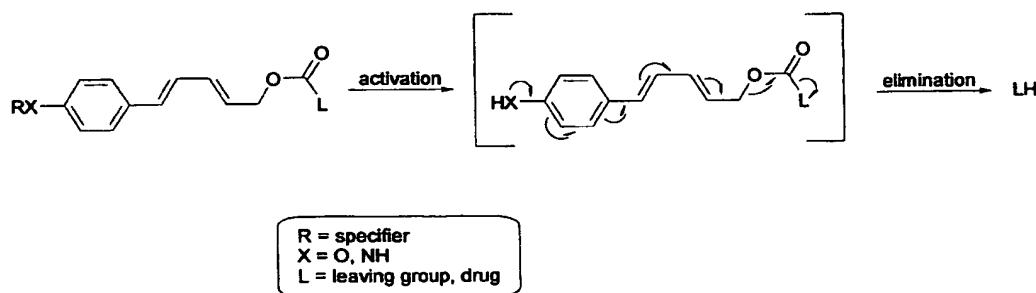
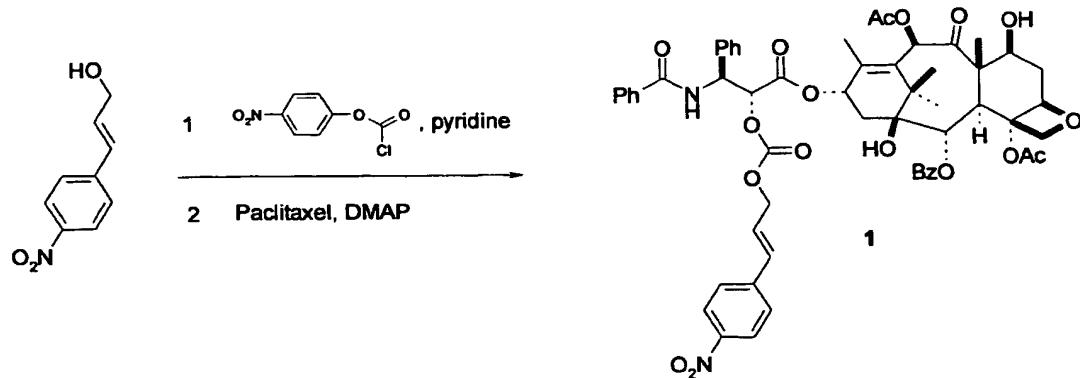
41. A pharmaceutical composition comprising a compound of any of claims 1 to 34.

**FIG. 1**

— (elongated spacer system) — drug

FIG. 2**FIG. 3**

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**FIG. 4****FIG. 5**

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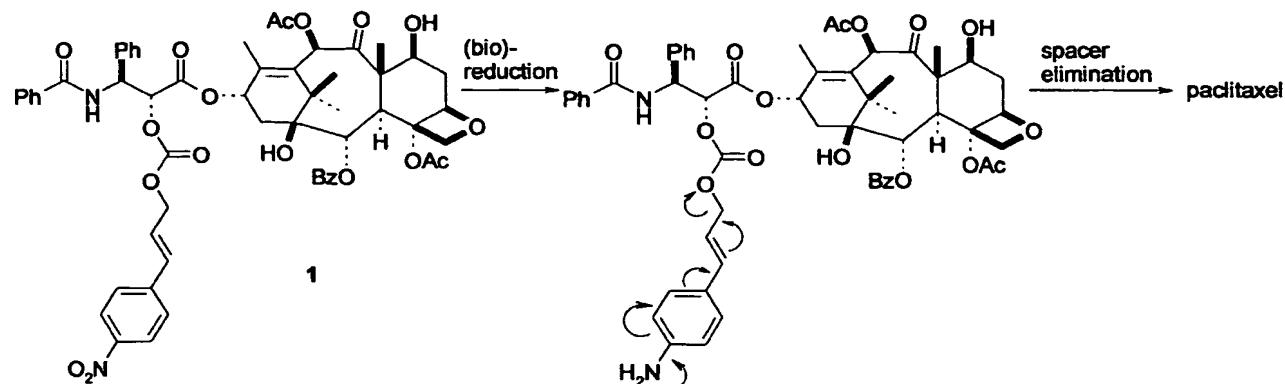


FIG. 7

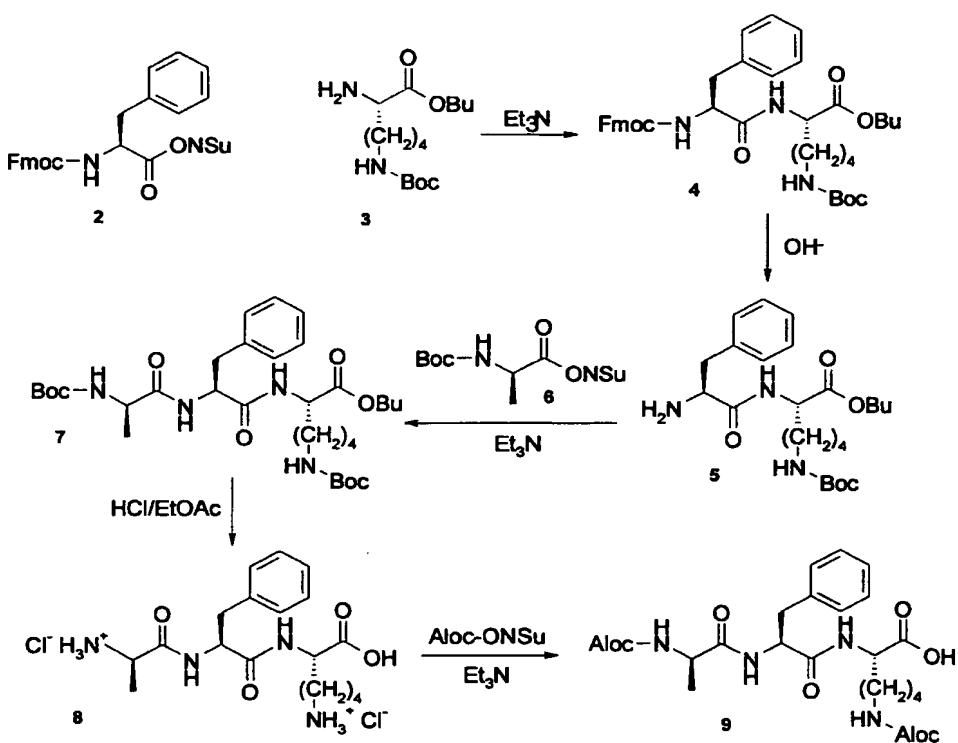
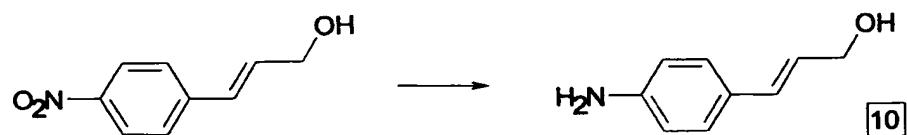
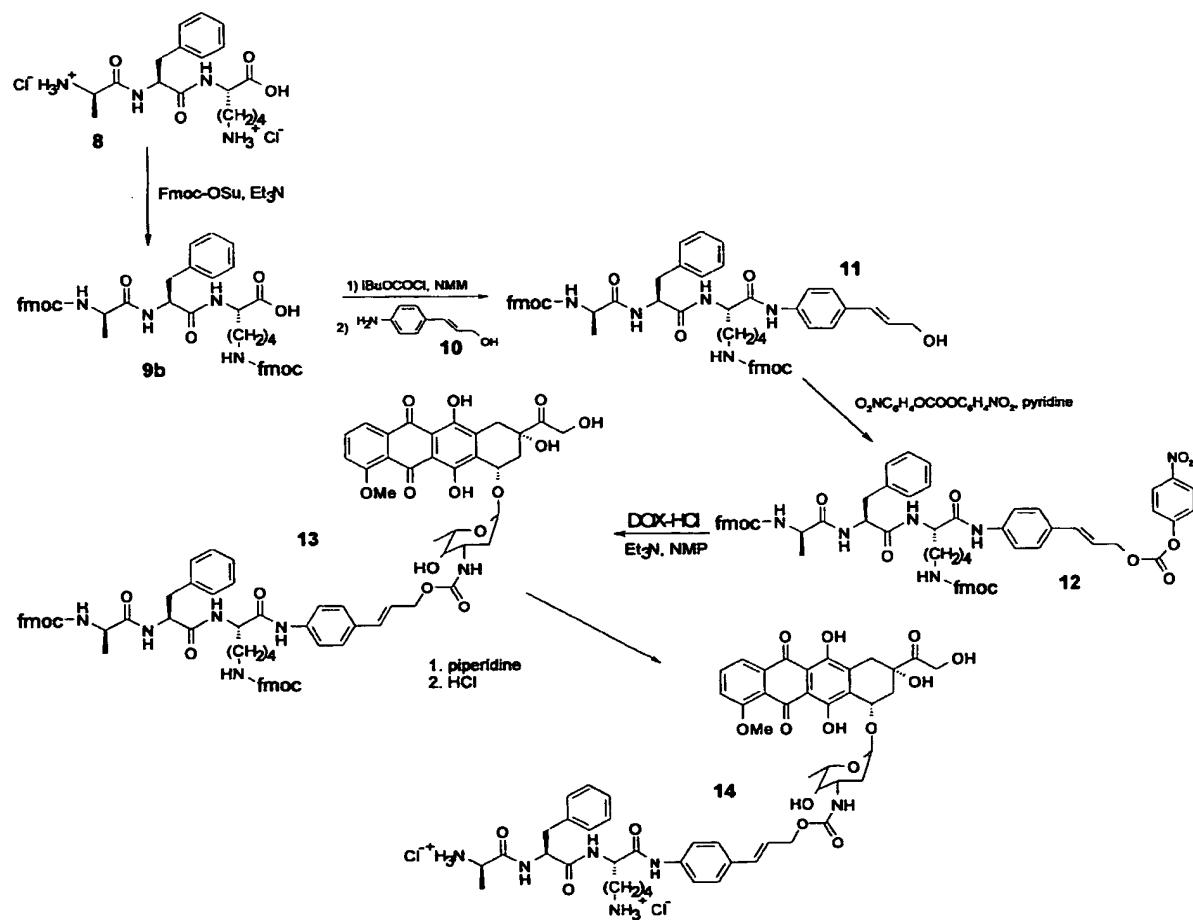


FIG. 8

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**FIG. 9****FIG. 10**

specifier — (electronic cascade spacer) — (electronic cascade spacer)_n — drug

FIG. 11

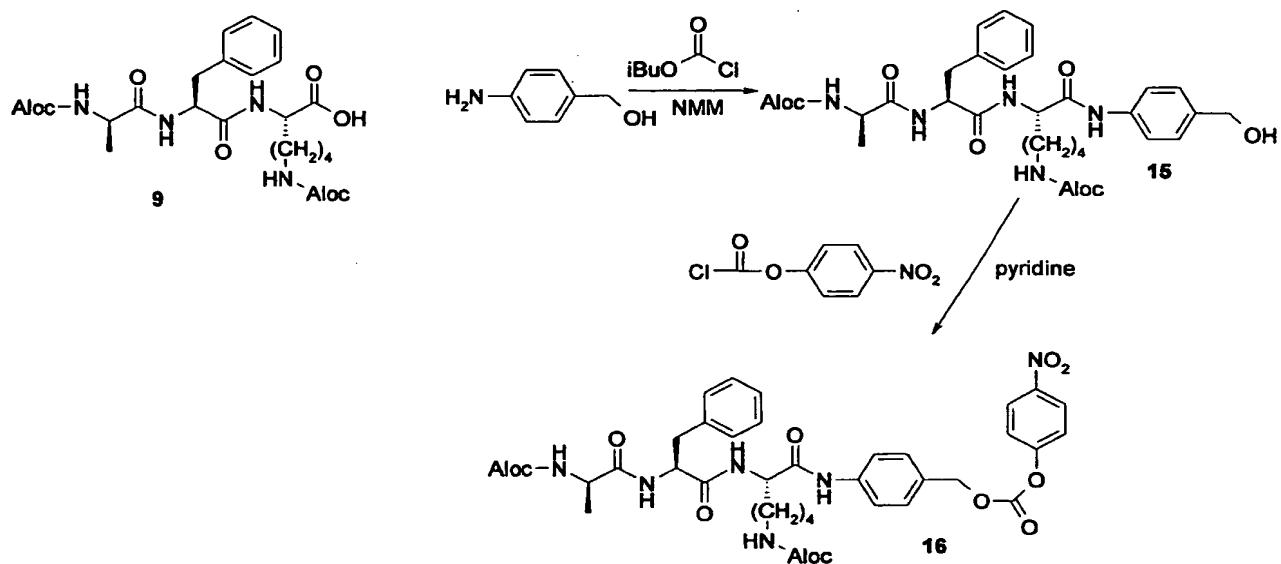


FIG. 12

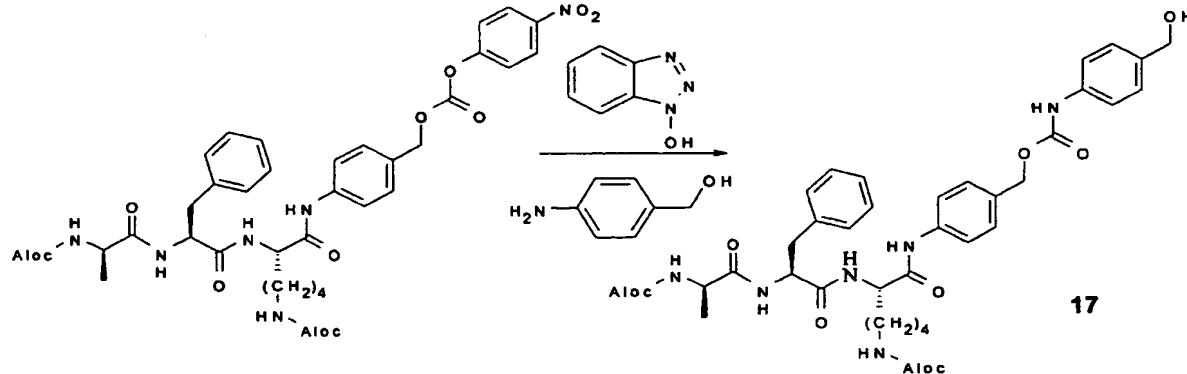


FIG. 13

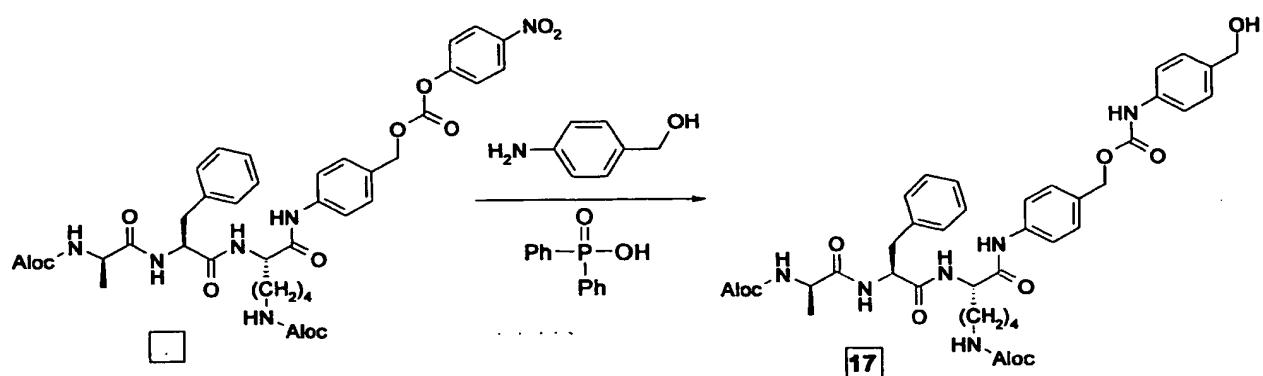


FIG. 14

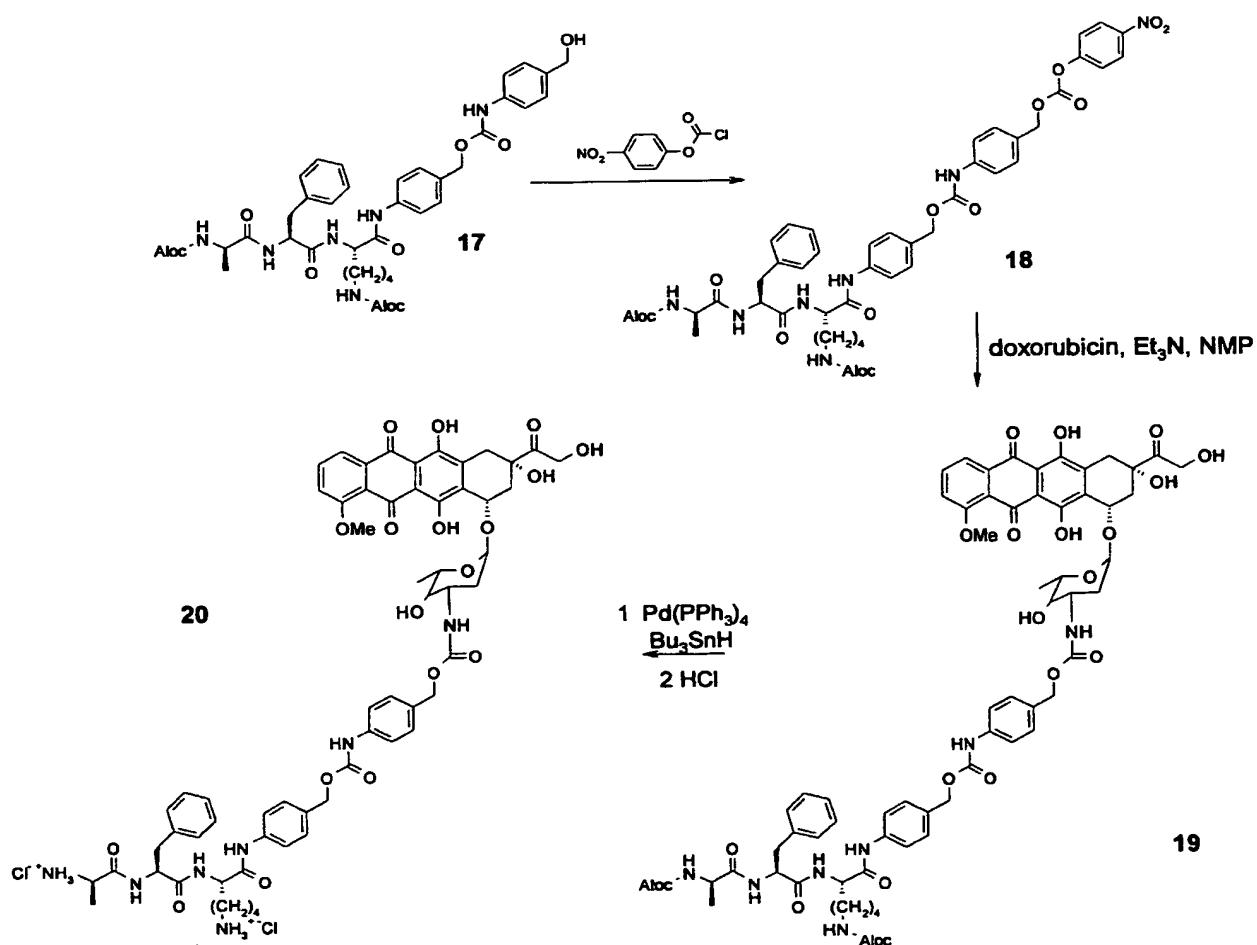


FIG. 15

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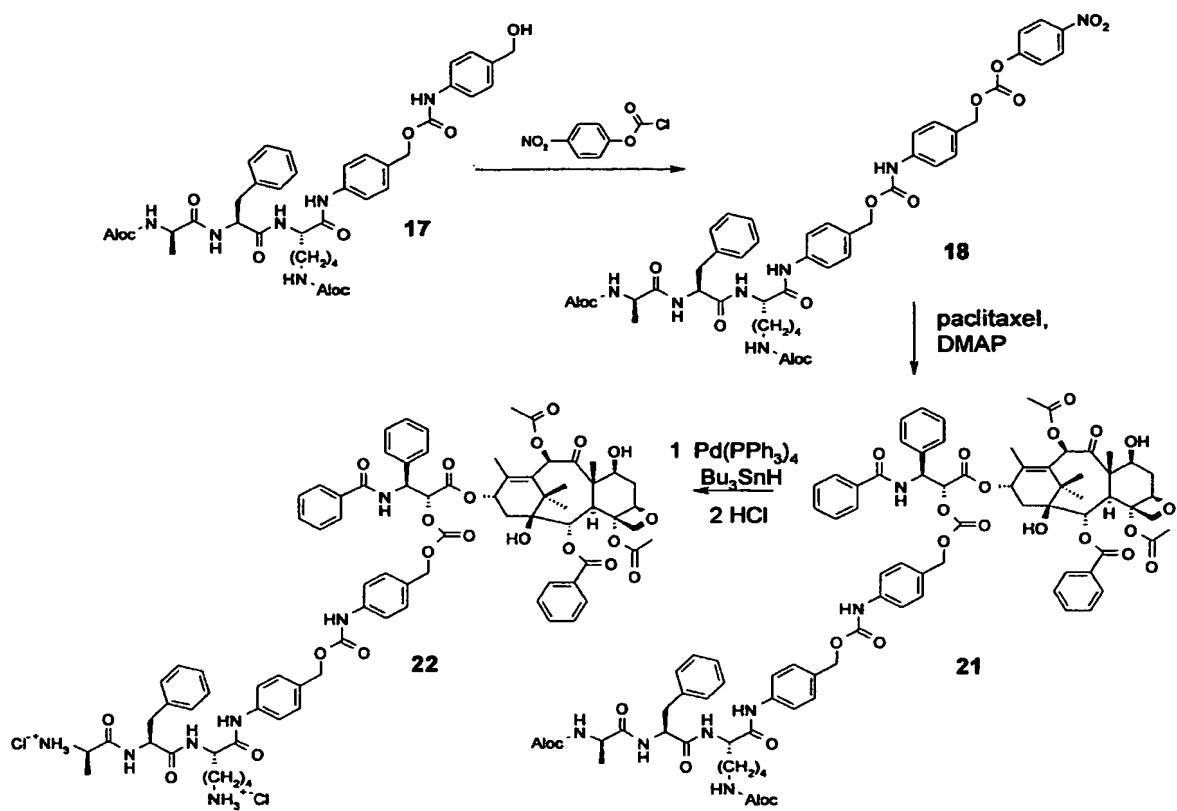


FIG. 16

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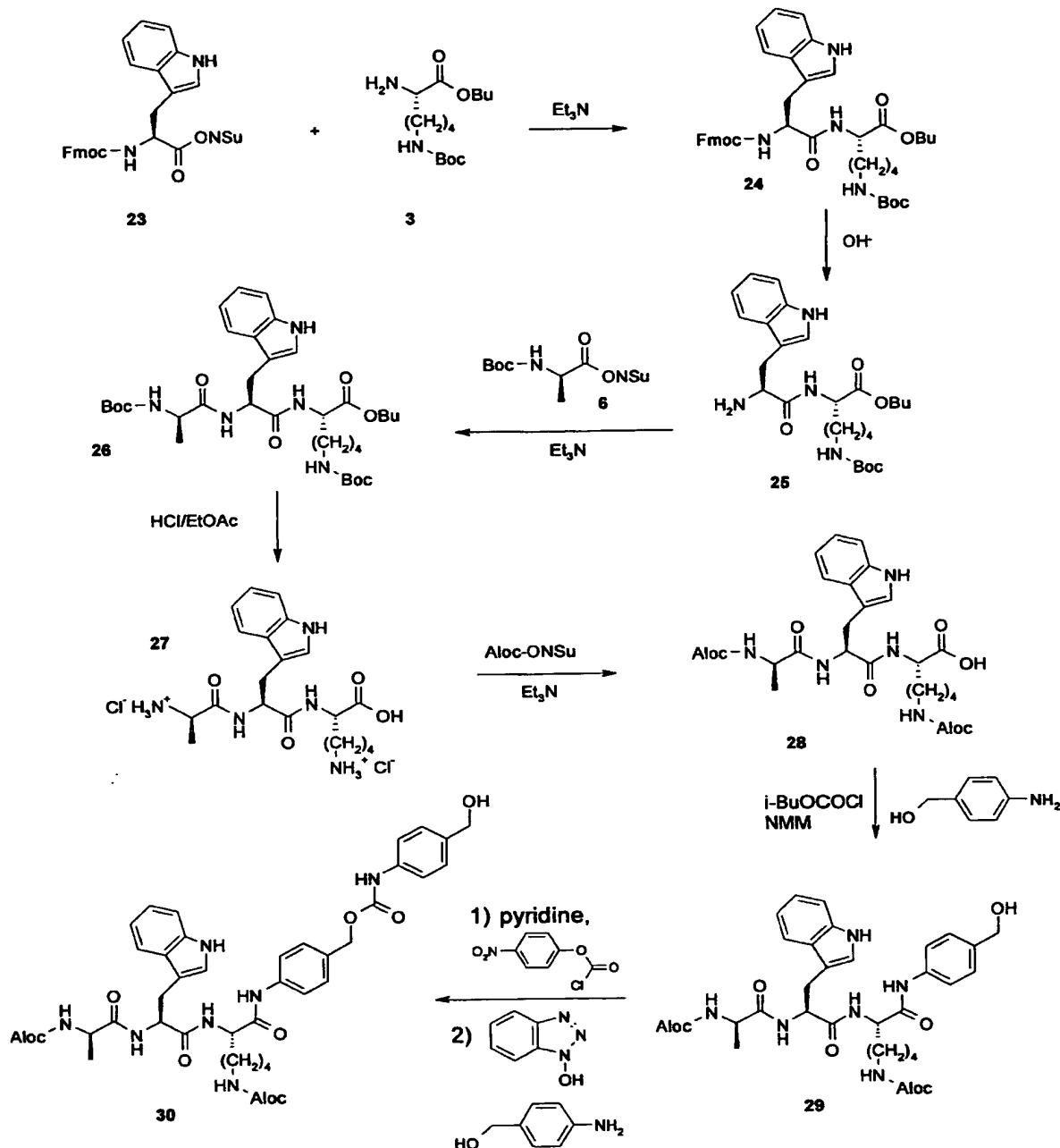


FIG. 17

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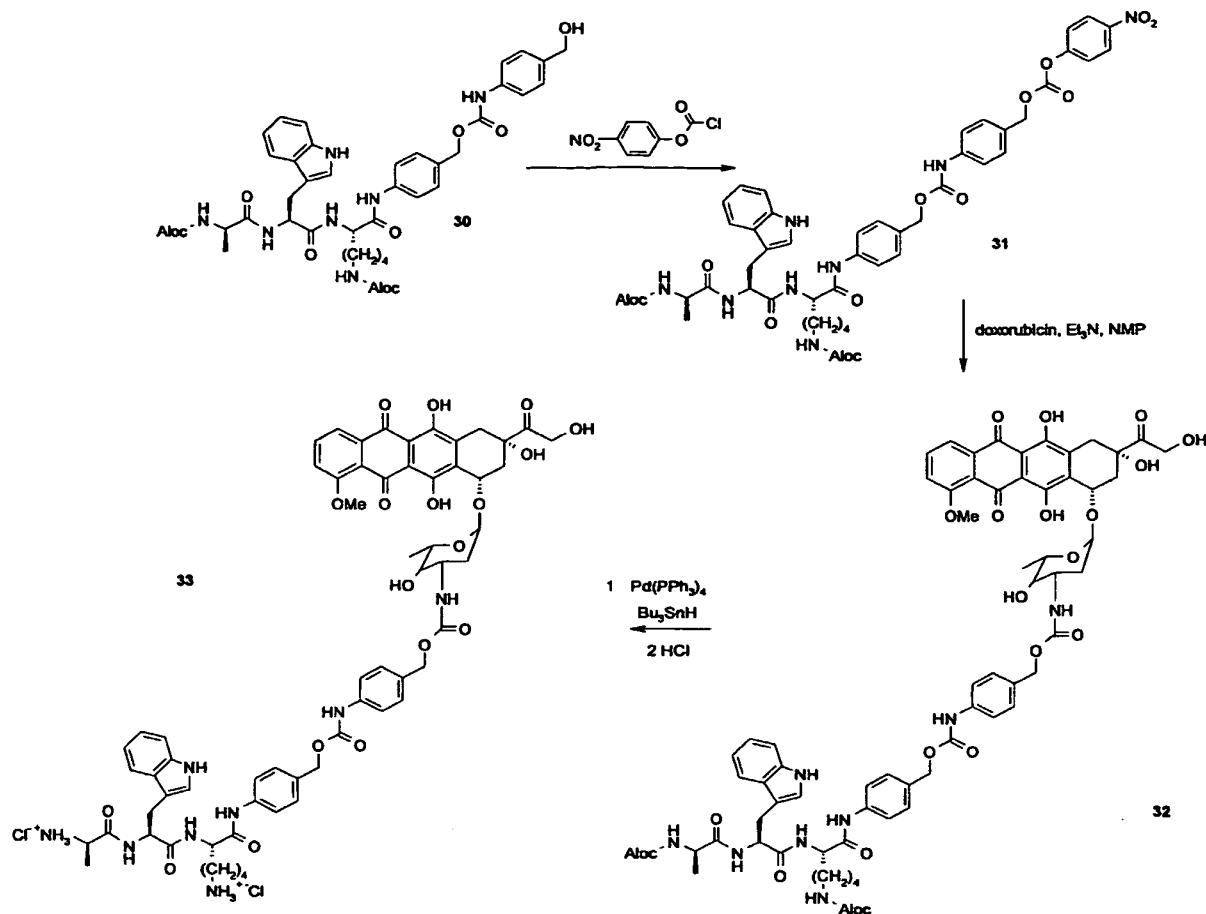
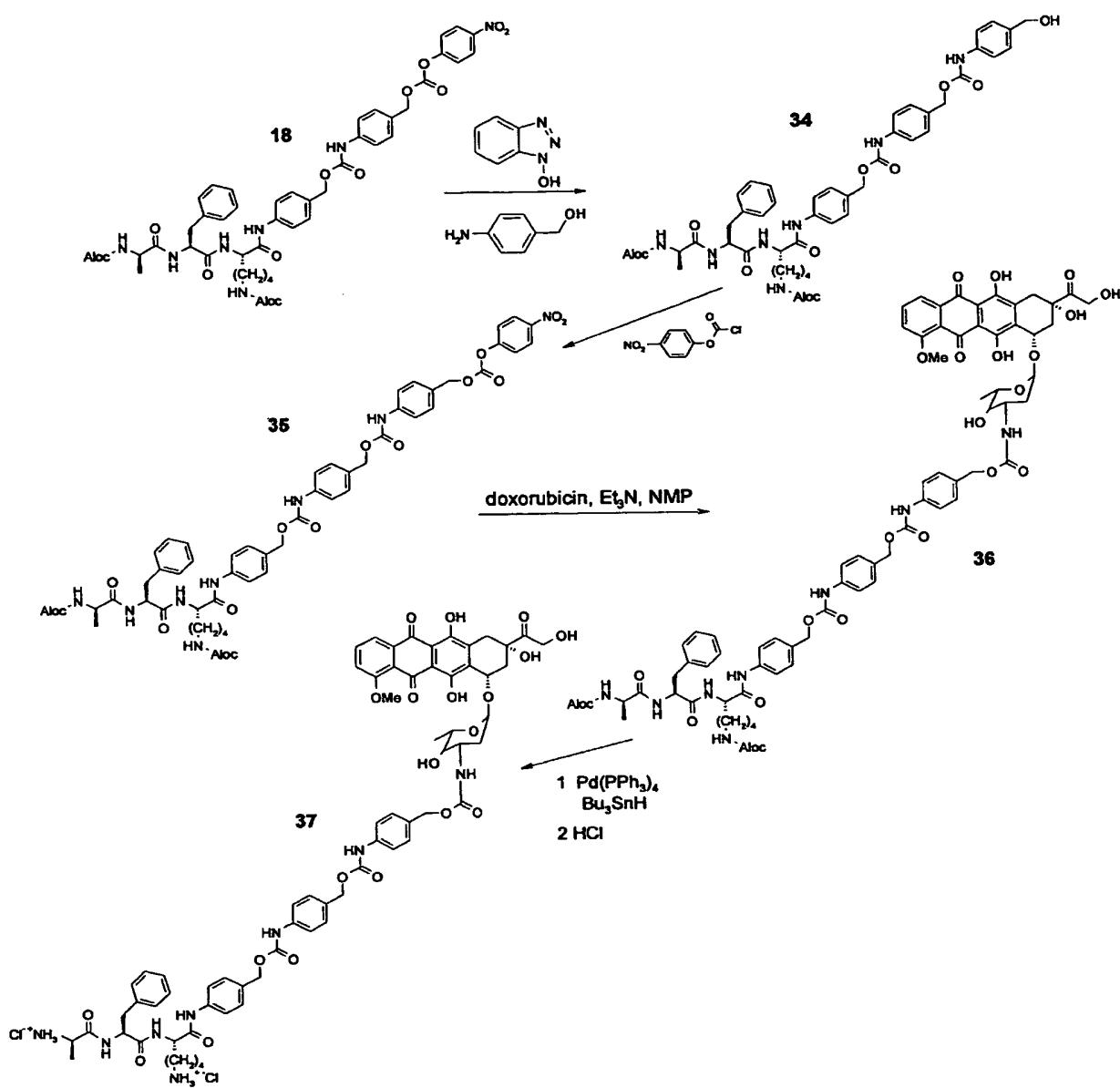


FIG. 18

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**FIG. 19**

specifier — (1,(4 + 2n)-electronic cascade spacer) — (cyclisation spacer) — 2'-O-paclitaxel

FIG. 20

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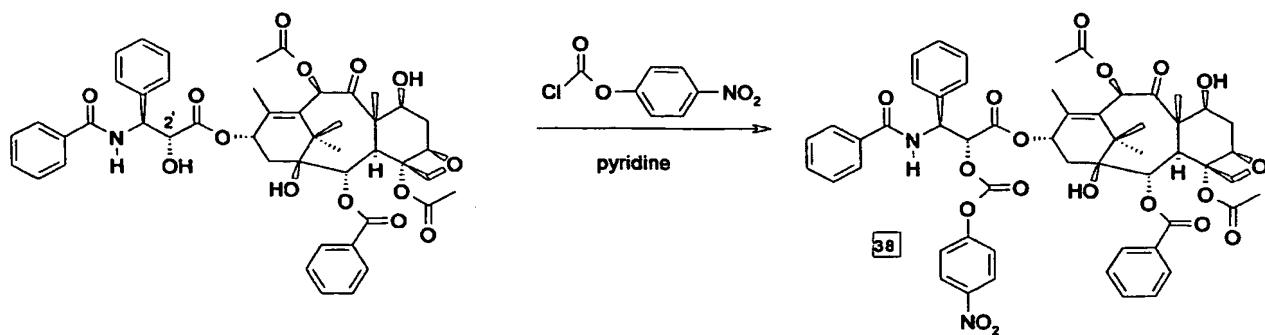


FIG. 21

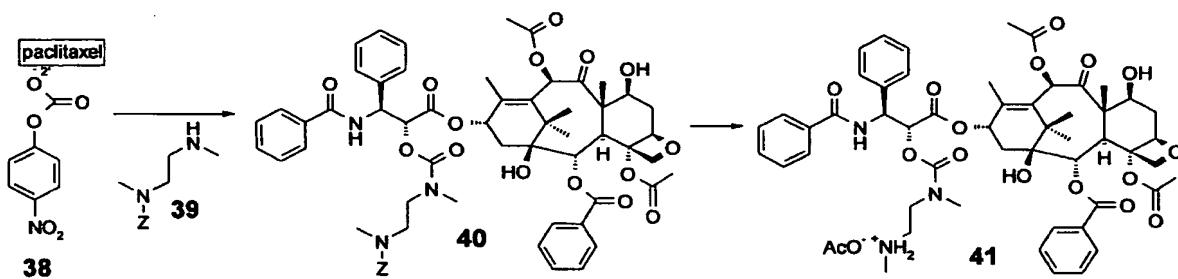


FIG. 22

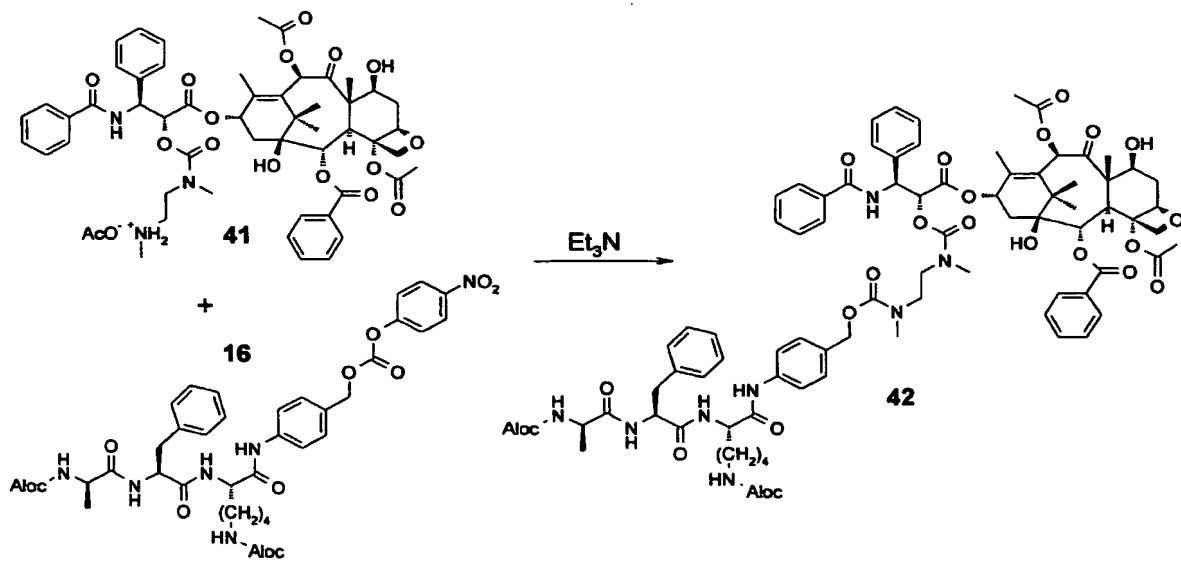


FIG. 23

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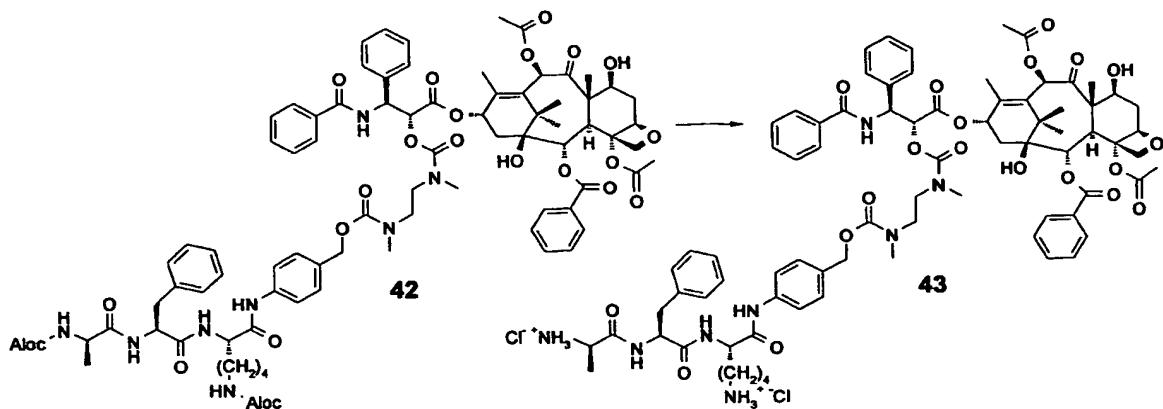


FIG. 24

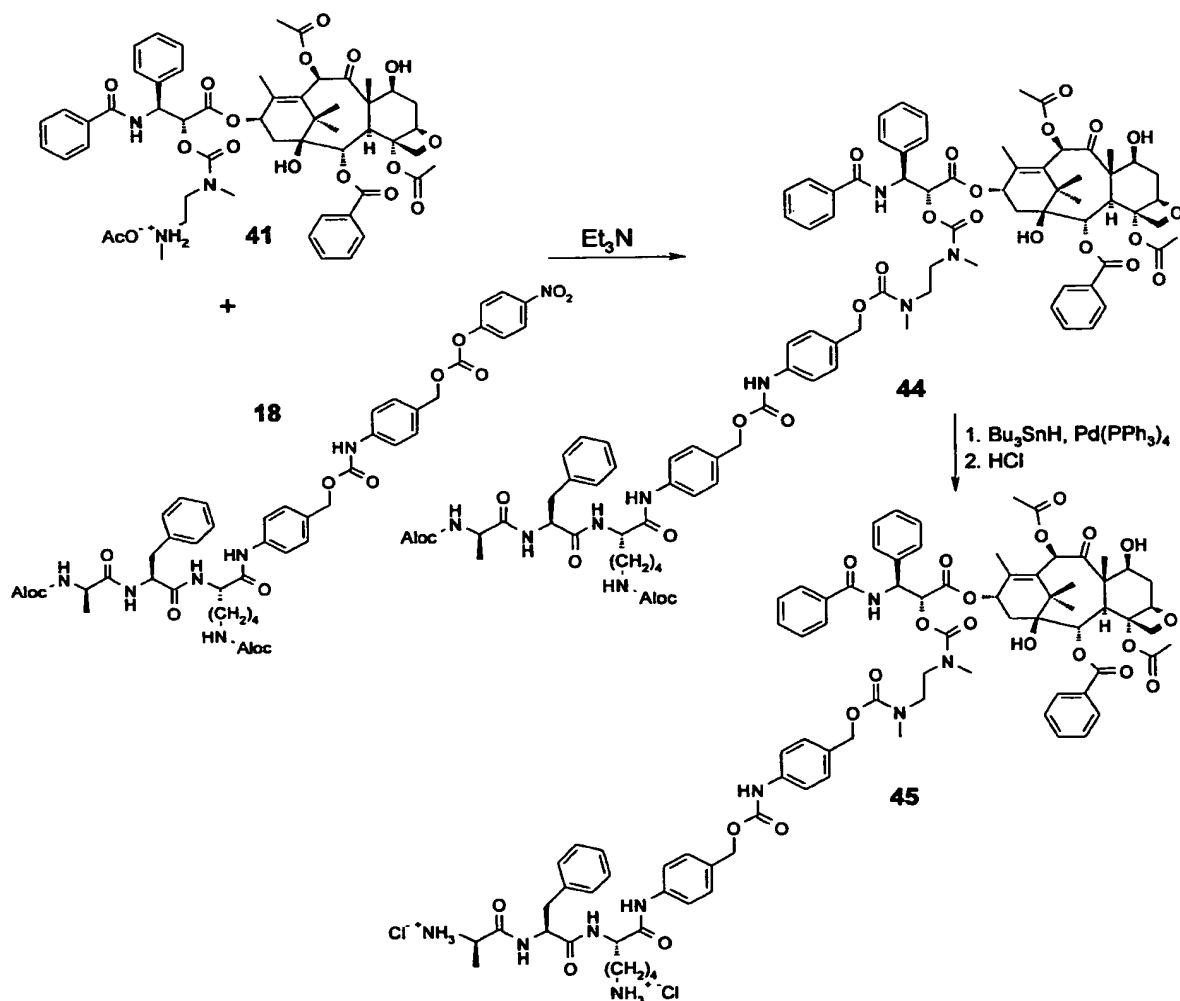
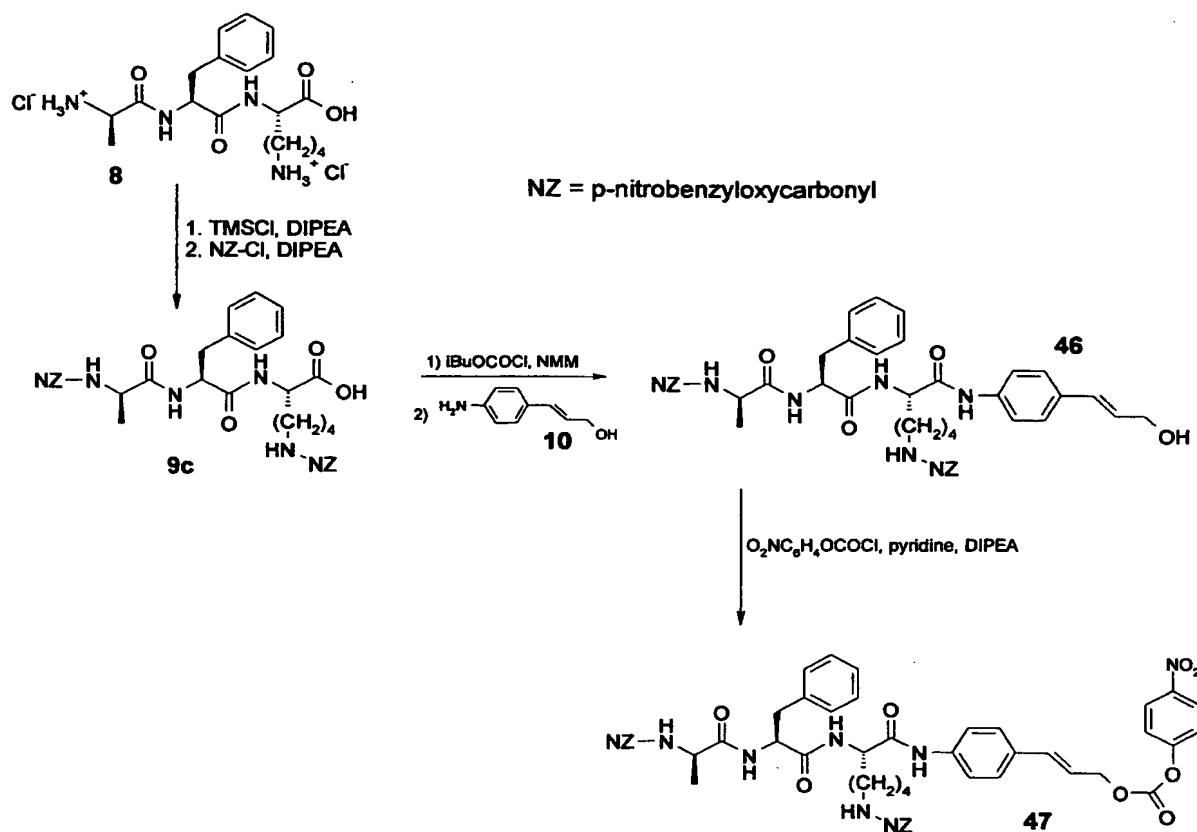


FIG. 25

**FIG. 26**

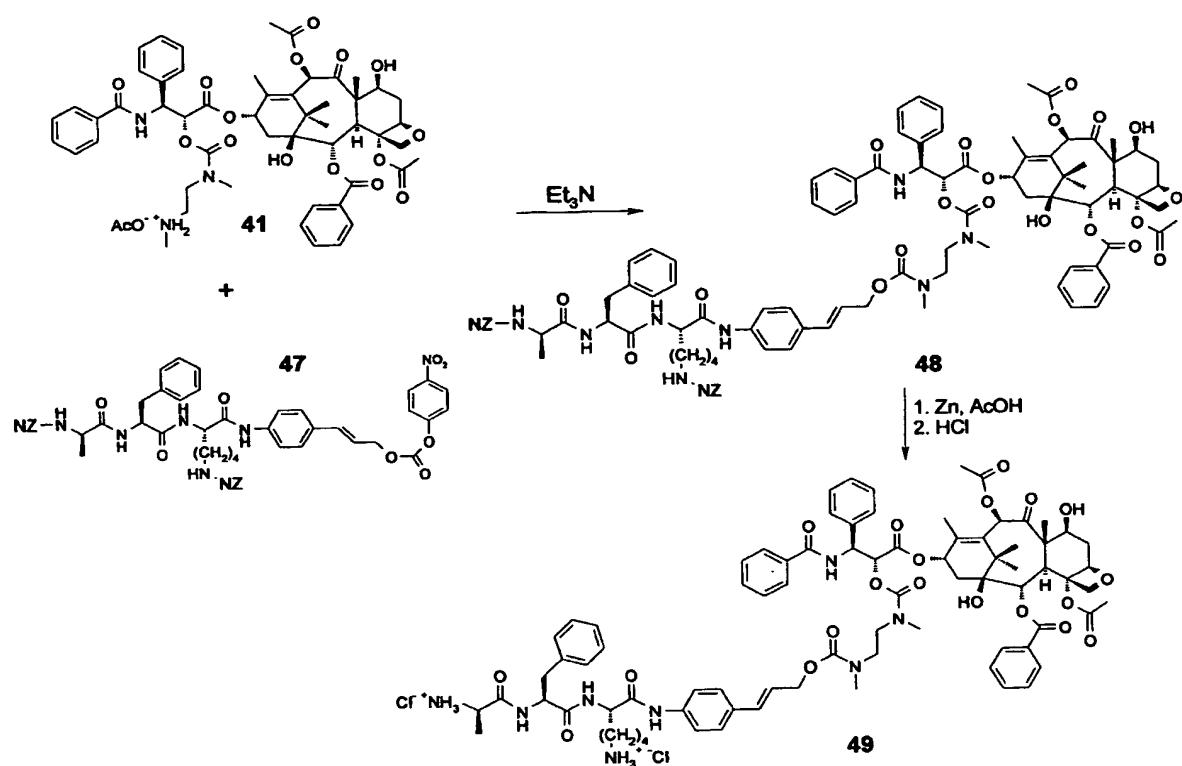


FIG. 27

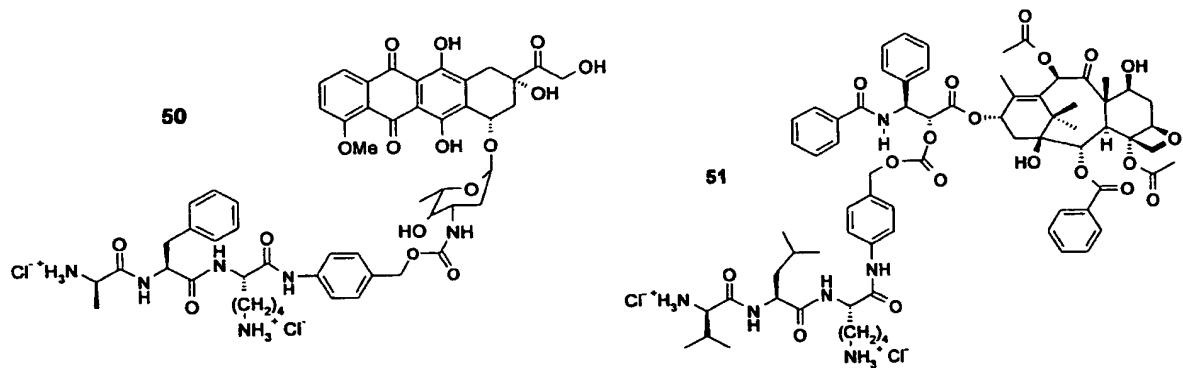


FIG. 28

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03591

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EMBASE, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE GROOT, FRANCISCUS M. H. ET AL: "Synthesis and Biological Evaluation of Novel Prodrugs of Anthracyclines for Selective Activation by the Tumor-Associated Protease Plasmin" J. MED. CHEM. (1999), 42(25), 5277-5283, XP002168683 Scheme 1,2,3 figure 1; examples; table 1 ---	1-41
X	WO 81 01145 A (UNIV ILLINOIS) 30 April 1981 (1981-04-30)	1-41
Y	page 19, line 15 - line 17; claims 1,18 page 22 ---	1-41
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

T later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

3 September 2002

Date of mailing of the international search report

16/09/2002

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Berte, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/03591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUBOWCHIK G M ET AL: "Cathepsin B-sensitive dipeptide prodrugs. 1. A model study of structural requirements for efficient release of doxorubicin" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 8, no. 23, 1 December 1998 (1998-12-01), pages 3341-3346, XP004143755 ISSN: 0960-894X scheme 2 , compound 26 table 1 ---	1-41
X	DUBOWCHIK, GENE M. ET AL: "Cathepsin B-sensitive dipeptide prodrugs. 2. Models of anticancer drugs paclitaxel (taxol), mitomycin C and doxorubicin" BIOORG. MED. CHEM. LETT. (1998), 8(23), 3347-3352, XP002168684 scheme 1 figure 1; table 1 ---	1-41
Y	WO 98 13059 A (SQUIBB BRISTOL MYERS CO) 2 April 1998 (1998-04-02)	1-41
X	claim 1 ---	1
X	EP 0 624 377 A (SQUIBB BRISTOL MYERS CO) 17 November 1994 (1994-11-17) formula 3 claims; examples ---	1-41
X	CHAKRAVARTY, PRASUN K. ET AL: "Plasmin-activated prodrugs for cancer chemotherapy. 2. Synthesis and biological activity of peptidyl derivatives of doxorubicin" J. MED. CHEM. (1983), 26(5), 638-44, XP002168690 scheme 1,2 ---	1-41
X	DE GROOT, FRANCISCUS M. H. ET AL: "Synthesis and Biological Evaluation of 2'-Carbamate-Linked and 2'-Carbonate-Linked Prodrugs of Paclitaxel: Selective Activation by the Tumor-Associated Protease Plasmin" JOURNAL OF MEDICINAL CHEMISTRY (2000), 43(16), 3093-3102 , XP002212034 page 3094, column 1, paragraph 4 -page 3094, column 2, paragraph 1 ---	1-41
		-/-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 691 371 A (DENNY WILLIAM ALEXANDER ET AL) 25 November 1997 (1997-11-25) claims; figure 1 -----	
P,X	DE GROOT, FRANCISCUS M. H. ET AL: "Elongated Multiple Electronic Cascade and Cyclization Spacer Systems in Activatable Anticancer Prodrugs for Enhanced Drug Release" JOURNAL OF ORGANIC CHEMISTRY (2001), 66(26), 8815-8830 , XP002212035 scheme 1-5 figures -----	1-41
P,X	DE GROOT FRANCISCUS M H ET AL: "In vivo efficacy of spacer-containing antitumor prodrugs of doxorubicin designed for specific activation by tumor-associated plasmin." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 43, March 2002 (2002-03), page 415 XP008007701 93rd Annual Meeting of the American Association for Cancer Research; San Francisco, California, USA; April 06-10, 2002, March, 2002 ISSN: 0197-016X abstract 2064 -----	1-41
P,X	DE GROOT FRANCISCUS M H ET AL: "Design, synthesis and initial biological evaluation of an alphavbeta3-integrin-targeted plasmin sensitive doxorubicin prodrug." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 43, March 2002 (2002-03), page 414 XP001051142 93rd Annual Meeting of the American Association for Cancer Research; San Francisco, California, USA; April 06-10, 2002, March, 2002 ISSN: 0197-016X abstract 2059 -----	1-41

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-41 relate to an extremely large number of possible compounds or methods. In fact, the claims contain so many variables and possible permutations that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely those compounds or methods have been searched recited in the examples and closely related homologous compounds.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/03591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 39 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No	
PCT/EP 02/03591	

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 8101145	A 30-04-1981		CA 1158557 A1 EP 0038357 A1 WO 8101145 A1	13-12-1983 28-10-1981 30-04-1981
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